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If a paper has not been seen in original it is safe to state 'Original not seen'.

Sources of information should be specifically acknowledged.

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FIG. 1. Heads of 'blue' wheats (No. 1, 2, 4) and dwarf hill-wheat (No. 3)



FIG. 2. Rachides, empty glumes, palea and kernels of 'blue' wheat (No. 1) and dwarf hill-wheat (No. 2) ($\times \frac{2}{3}$)

ORIGINAL ARTICLES

THE CYTOLOGY OF 'BLUE' WHEAT HYBRIDS

BY
T. C. CHIN
AND
C. S. CHWANG

Department of Agronomy, College of Agriculture, University of Nanking

(Received for publication on 8 January 1942)

(With Plate XXVI and 21 text-figures)

WHEAT is the most popular crop in north China. It is next in importance to rice in the southern and south-western provinces of the Yangtze valley. Practically all of the important cultivated varieties belong to *T. vulgaris*. The information regarding the existence of *Emmer* wheats and other species of *vulgare* group is rather meagre. As reported by various workers [Osono, 1935, 1; King, 1934; Shen, 1937] varieties of *T. durum* have been grown in some localities in Yunnan, Hupeh, and Sinkiang and those of *T. rigidum* in Sinkiang, Honan and Kansu. It was not until our recent migration to Szechwan, after the Sino-Japanese hostilities, that we realized the significance of the so-called 'blue' wheat of Szechwan as a distinct group of *Emmer* wheats.

Many different local names are given to them and 'blue' wheat is the most popular and collective one. It is so named because the bloom or wax deposited on the surface of the green tissues appears bluish in colour. Another closely related form of *Emmer* wheats in Szechwan is known as dwarf hill-wheat. It differs primarily from the 'blue' wheat in the absence of waxy substance, in a stouter plant stature, and in a somewhat different head type; yet both yield crops of extraordinarily poor quality. Information regarding the habitats of these wheats, as reported from various sources, seems conflicting. In some places they are suited to dry regions or mountain slopes, while in other places they grow well on moist, damp soils, such as river valleys. Therefore, opinions differ as to the botanic position of these wheats. Some state them to be *T. turgidum* and others *T. durum*. So far as the writers are aware, no cytological work on these two forms of *Emmer* wheats has yet been done. This paper reports an inquiry into the identification of these wheats by means of cytological studies on various 'blue' wheat hybrids.

GENERAL DESCRIPTION

A. *Geographical distribution.* 'Blue' wheat is found to be cultivated as a winter crop in various districts of western and north-western Szechwan. Its cultivation in this province goes back a long way and no exact evidence of the time of its introduction is available; but the limited degree of morphological and physiological differentiations or variations suggests that its introduction might not be of remote antiquity.

The dwarf hill-wheat, as indicated by the name, is commonly cultivated along the hillsides of the mountainous regions of western Szechwan. Although

no actual data concerning the acreage or production of these two forms of wheats in Szechwan is available, it has been estimated that they probably constitute about 5 per cent of Szechwan's wheat crop.

B. *General morphology.* In Szechwan where winters are mild 'blue' wheat (including dwarf hill-wheat) is customarily fall-sown. Within our collection there appear a number of varieties with different seedling habits. Among 249 strains of 'blue' wheat studied, 210 strains assume the upright, spring habit; 21 prostrate, and 18 semi-prostrate type. All the nine strains of dwarf hill-wheat possess upright seedlings. They seldom stool or tiller. The number of tillers varies from four to seven per plant. The leaves are usually broad and smooth but possess a peculiar whitish green colour with an extremely harsh cuticle. As a rule, the culms are somewhat taller (except dwarf hill-wheat) than those of common wheats, ranging from 0.915 to 1.515 mm. and hence the tonnage of green material produced per unit area tends to be great. This is, perhaps, one of the reasons why in some localities in Szechwan it is preferred for hay. The straw is of medium stiffness, with a dull, thick striate surface.

So far we have not observed any 'blue' wheat which is beardless. The ears average 9 cm. in length and 1.5 cm. in width, possessing 20 or more spikelets. Three to five florets are found in each spikelet and generally three are fertile. Most varieties have short, thick, compact heads which are laterally compressed and more or less rectangular in cross section. The rachis is tough, smooth, but copiously fringed along its edges with white hairs and bears a frontal tuft of similar hairs at the base of each spikelet, reaching approximately a length of 3-4 mm. The empty glumes are white, pubescent on both surfaces and prominently and sharply keeled at the base. The awns are stout, rough, grayish white in colour, triangular in section, erect and projecting upward.

The grains are usually amber or yellow, but occasionally pale red in colour, large, broad and plump with a high dorsal arch or hump behind the embryo. The endosperm is opaque and starchy although in a few varieties it is quite hard and vitreous. A majority of varieties possess an intermediate condition, the texture is rather hard while opaquely white and non-translucent assuming a porcelain-like structure rather than starchy fractures. However when they are grown under more or less humid conditions they are almost starchy.

As already pointed out above, 'blue' wheat is characterized by its waxy appearance. All of our collection except nine are waxy; the bloom covers all the plant parts. Those possessing no waxy bloom (here designated as 'dwarf hill-wheat') have very short, thick-walled culms with light yellowish green foliage. The ears are fully bearded, with medium length and density. With the exception of the ear type, the morphological characteristics and the growth habit suggest that the so-called dwarf hill-wheat is most probably a related form of *T. pyramidale*.

C. *Cultural characteristics, quality and disease reactions.* As 'blue' wheat tillers less than the common winter wheat, seeding is, therefore, somewhat heavier than for common wheats. It grows rapidly and the heavily bearded heads tend to have a nodding habit; this together with the

ically bloomy appearance makes them look like a fish tail and thus the 'fish-tail' wheat is given by the farmers in certain localities.

The vigorous vegetative growth is accompanied by a delay of development. Heads about a week later than the late varieties of common winter wheats consequently matures later. Such an inherently long period of growth would, in part, account for its high yielding capacity. But its tall growth and late ripening may be more readily subject to attacks by birds than ordinary wheat. The straw is moderately stiff so that it is possible for birds to perch upon it and, because it is tall, it ranges above all other wheats and is more conspicuous to the eyes of the birds. It requires a moderately hot season for satisfactory growth and thus it is more drought-tolerant. The hardness of the kernels is conditioned by the environmental factors.

As shown in our data on nitrogen determination (Table I) it may be said in general that 'blue' wheat tends to contain more protein than other wheats commonly cultivated in Szechwan, especially when the environment is favourable. Yet the baking quality of 'blue' wheat and dwarf hill-wheat flours is rather poor. Their gluten is less elastic than that of common wheats and their swelling capacity and the gas-retention capacity are unexpectedly low. They yield a heavier and stiffer dough with comparatively less water than other flours. Probably the gluten of 'blue' wheat as well as its starch differs physico-chemically from that of other wheats. This is the reason why flour from 'blue' wheat is primarily used in the baking of cakes and biscuits.

TABLE I
Protein content of wheats

(1940-41 crop from the University Farm, Chengtu, China)

Varieties	Moisture (per cent)	Protein (per cent)
'blue' wheat	15.99	11.79
'blue' wheat	15.28	12.20
'blue' wheat	16.86	9.37
dwarf hill-wheat	17.32	10.89
2905 (common wheat)	14.62	10.60
4197A (common wheat)	15.51	10.20

* Improved varieties of the University Farm

None of the 'blue' wheat strains tested escapes from the infection of stripe, *P. glumarum*, which is rather serious in the west Szechwan plain, yet none of them reaches a severe stage of infection. They are more or less susceptible to loose smut, *U. tritici*, in most localities and, stinking smut, *U. tritici*, in certain districts. Scab, caused by *Gibberella saubinetii*, is of common occurrence in Szechwan but, according to the writers' experience, these 'blue' wheats when sown in Kweichow, (a neighbouring province south of Szechwan) where wheats are generally harvested one month later than in Szechwan, suffer rather seriously from the attack of scab fungus. It is noted that the compact spike of 'blue' wheat with heavy tufts of hairs on each rachis joint slows down the rate of drying after rains or heavy dews and consequently facilitates the attack of the pathogene. Wu [1940] tested

for the resistance of 'blue' wheat varieties to flag smut, *Urocystis tritici*, by artificial inoculation and found that none of the varieties tested was not immune. They are generally more susceptible, particularly those of soft varieties, damage by flour moth and grain weevil than the common bread wheats under storage conditions.

Cytological observations reveal that the typical 'blue' wheat as well as the dwarf hill-wheat possesses normally 14 bivalents in meiosis (Fig. 1). The peculiarities of 'blue' wheat as a distinct group and its doubtful botanical position appear to justify a study of these subjects on which little literature is available. The present discussion rests primarily upon as yet incomplete information on the cytological behaviour of the hybrids between 'blue' wheat and other known species of *Triticum*.

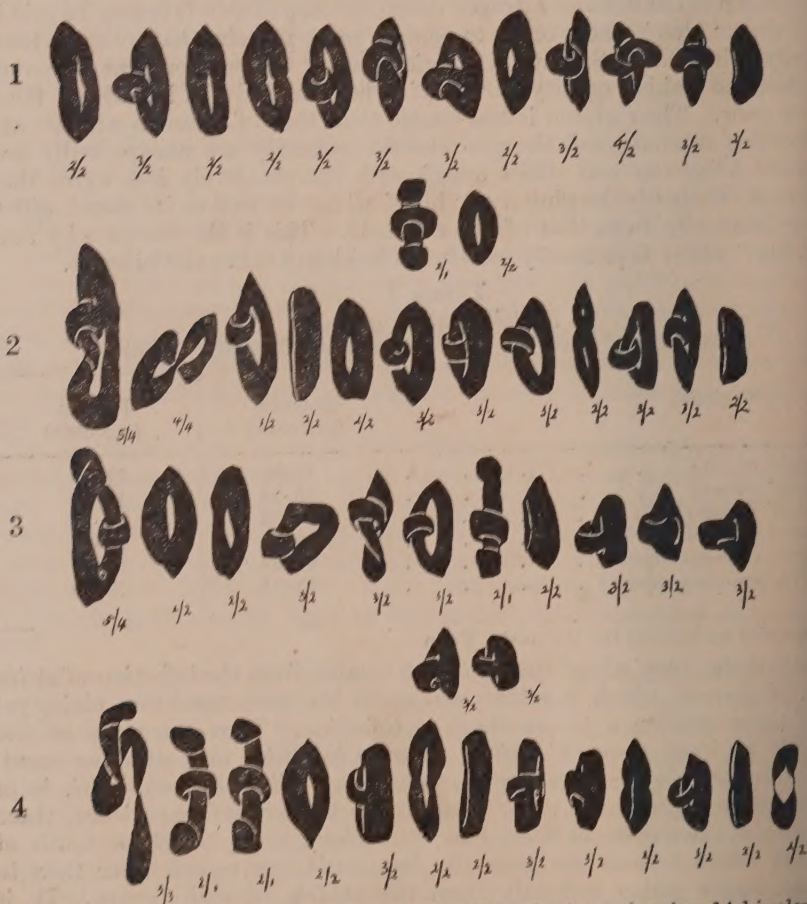


FIG. 1. Chromosomes of metaphase I in 'blue' wheat showing 14 bivalent
 FIG. 2. Two rings of 4 and 10 bivalents in *T. durum* × 'blue' wheat
 FIG. 3. A ring of 4 and 12 bivalents in *T. turgidum* × 'blue' wheat
 FIG. 4. A chain of 4 and 12 bivalents in *T. pyramidale* × 'dwarf hill-wheat'

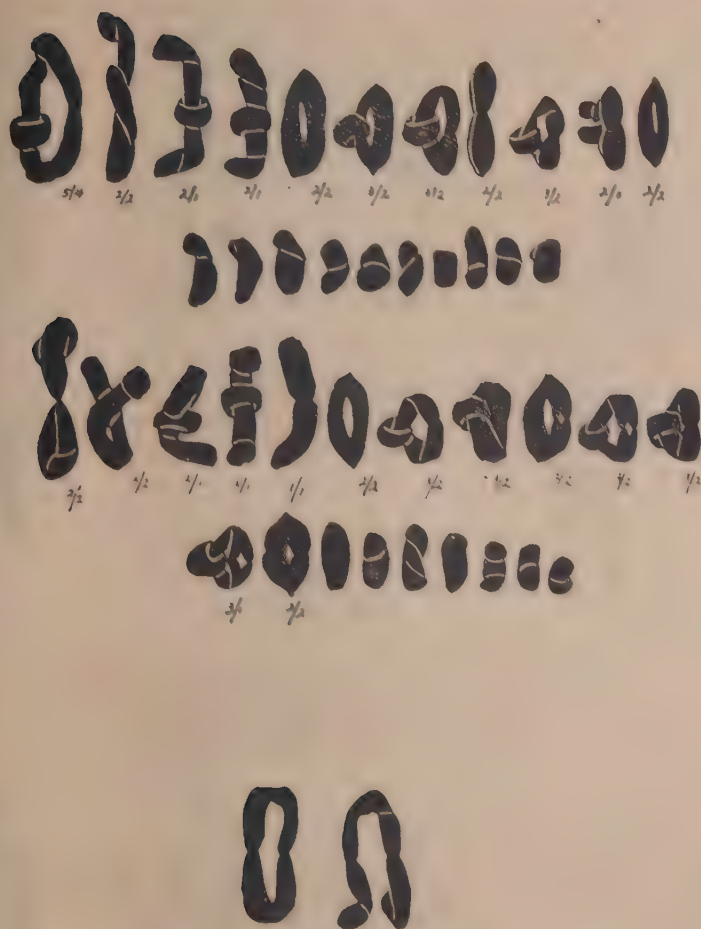


FIG. 5. Metaphase I in *T. vulgare* × 'blue' wheat showing
1 (IV) + 1 (III) + 9 (II) + 10 (I)

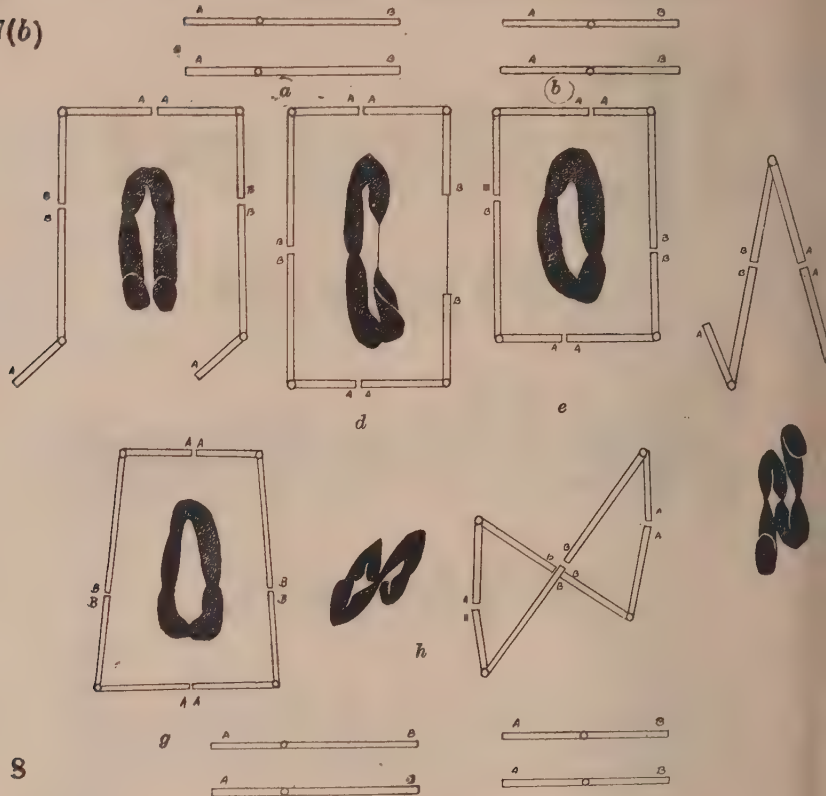
FIG. 6. Metaphase I in *T. sphaerococcum* × 'blue wheat' showing
2 (III) + 11 (II) + 7 (I)

FIG. 7 (a). Association of 4 in *T. durum* × 'blue' wheat. All the four
members possess sub-median centromeres

MATERIAL AND METHODS

The material used in the crosses includes *T. durum* Desf. (var. Iumillo) × *T. turgidum* L., *T. pyramidale* var. *recognitum* Perc., *T. vulgare* (NK 2905) × *T. durum*, *T. sphaerococcum* Perc. and 'blue' wheat and dwarf hill-wheat collected in Szechwan.

7(b)



8

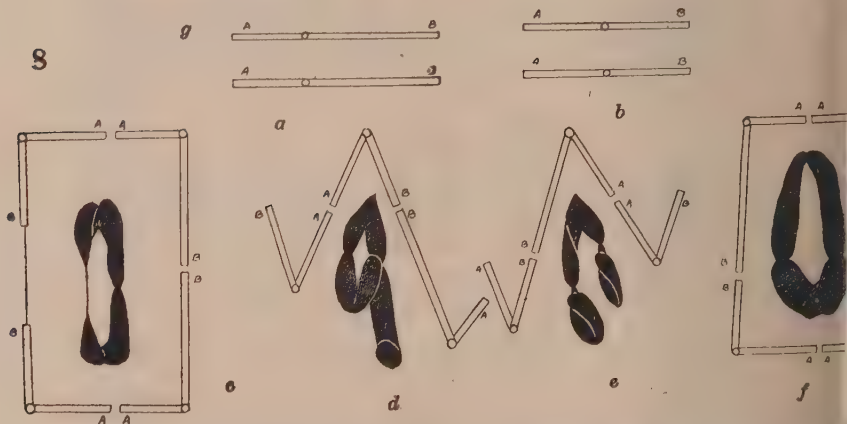


FIG. 7 (b). Configurations of *T. durum* × 'blue' wheat; a and b, two pairs of diagrammatic chromosomes taking part in the multiple configurations; one pair with median centromeres and the other pair with sub-terminal centromeres; c—h, associations of four found from different individuals. They are composed of the same chromosomes.

FIG. 8. Configurations of *T. turgidum* × 'blue' wheat; a and b, two pairs of diagrammatic chromosomes taking part in the multiple configurations; one pair with median centromeres and the other pair with sub-terminal centromeres; c—f, multiple associations found from different individuals. They are composed of the same chromosomes. The two sets of chromosomes in the two hybrids are morphologically similar.

The crosses were made in 1940 and the slides were prepared in 1941. Smears were fixed in La Cour 2BE and were stained by Newton's gentian violet iodine method. Drawings were made with the aid of camera lucida.

RESULTS

A. Tetraploid hybrids

1. *T. durum* \times B. W., ('blue' wheat). Besides the bivalents there are two rings of four chromosomes in this hybrid. One of the rings is composed of four chromosomes with sub-medial centromeres (Fig. 7a), and the other comprises two chromosomes with median centromeres and two with sub-medial centromeres (Figs. 2 and 7b). The average number of associations of four is 0.9 (Table II).

Bridges (Fig. 9) are observed in the first anaphase. The percentage of bridges is equal to 4.88 and the coefficient of hybridity is 0.00161.

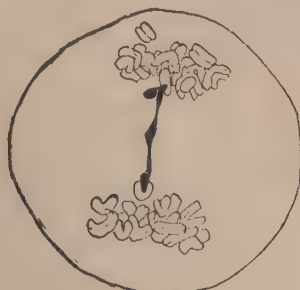


FIG. 9. First anaphase bridge in *T. durum* \times 'blue' wheat

TABLE II
Configurations of tetraploid hybrids






Configurations and No. of X-t a	Bivalents					Multiple configurations				Number of cells observed
						Association of four			Asso- ciation of 3+1	
						5.4	4.4	3.3		
	4.2	3.2	2.2	2.1	1.1	5.4	4.4	3.3	2.2	
<i>Durum</i> × B. W. (per cent)	..	54	182	1	9	2	11	4	..	20
	..	21.7	74.2	0.4	3.7
<i>Turgidum</i> × B. W. (per cent)	4	119	78	3	..	1	1	..	8	16
	2.0	58.3	38.2	1.5
<i>Pyramidale</i> × D. H. W. (per cent)	..	74	38	5	5	..	4	5	..	10
	..	60.6	31.1	4.1	4.1

TABLE III
Distribution of chiasmata of tetraploid hybrids

Hybrids	Mean No. of bivalent	X-ta per bivalent	X-ta per potential bivalent	Total X-ta per cell	Terminal X-ta per cell	Coefficient of terminalization
<i>Durum</i> × B. W.	12.30	2.18	2.15	30.15	27.30	0.905
<i>Turgidum</i> × B. W.	12.75	2.62	2.50	35.00	26.81	0.766
<i>Pyramidale</i> × B. W.	12.20	2.57	2.46	34.40	26.50	0.770

The mean number of bivalents per cell is 12.30. The number of chiasmata per bivalent is 2.18 and that for each potential bivalent is 2.15. The mean number of chiasmata is 30.15 and the coefficient of terminalization, 0.905.

2. *T. turgidum* × B. W. There is only one multiple configuration which is most frequently an association of three and occasionally an association of four (Table II; Figs. 3 and 8). In the latter there are two chromosomes possessing median centromeres and two possessing sub-median centromeres.

Both the first and the second anaphase of this hybrid are normal. The average number of bivalents per cell is 12.75. The mean frequency of chiasmata per bivalent is 2.62 and that for each potential bivalent, 2.50. The mean frequency of chiasmata per cell is 35.00. The coefficient of terminalization is 0.766.

3. *T. pyramidale* × D. H. W. (dwarf hill-wheat).* There is only one association of four chromosomes (Fig. 4). The number of chiasmata per potential bivalent is 2.46 and that for each individual cell is 34.40. Out of 48 first divisions there are two single bridges (Fig. 11) in the same cell and five single bridges (Fig. 10) in 146 second divisions forming a percentage of 3.61. The coefficient of hybridity is 0.00105 showing that dwarf hill-wheat differs from *T. pyramidale* by a small portion of inversion.

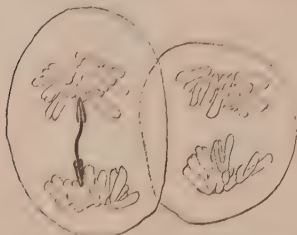


FIG. 10. Second anaphase bridge in *T. pyramidale* × dwarf hill-wheat

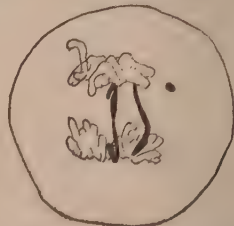


FIG. 11. Two single first anaphase bridges in *T. pyramidale* × dwarf hill-wheat

* The cross was made by C. K. Chan, to whom the writers are indebted

Pentaploid hybrids

1. *T. vulgare* (NK 2905) \times B. W. There are two multiple configurations, association of four and an association of three (Tables IV and V; Fig. 5). Average number of bivalents per cell is 11.9 and the mean number of valents per cell is 8.8. The average number of chiasmata per potential bivalent and that for each cell are 2.14 and 30.40 respectively. The coefficient of terminalization is 0.77. The percentage of bridges in the first division (Figs. 12 and 13) is 8.33 and the coefficient of hybridity is 0.00278.



FIG. 12. First anaphase bridge in *T. vulgare* \times 'blue' wheat



FIG. 13. First anaphase bridge and a fragment in *T. vulgare* \times 'blue' wheat

TABLE IV

Configurations of pentaploid hybrids

Configurations and No. of X-ta	Bivalents				Multiple configurations				No. of cells observed
	4	3	2	1	Association of four			Association of three	
					5	4	3	2	
<i>ere</i> × B. W.	2	58	43	16	1	1	1	4	10
<i>erococcum</i> × B. W.	2	20	31	5	..	1	1	3	5

TABLE V

Distribution of chiasmata of pentaploid hybrids

Hybrids	Average No. of bivalents per cell	Average No. of univalents per cell	X-ta per potential bivalent	X-ta per cell	Coefficient of terminalization
<i>T. vulgare</i> \times B. W.	11.9	8.8	2.14	30.40	0.77
<i>Hordeum</i> \times B. W.	11.6	8.4	2.11	29.60	0.68

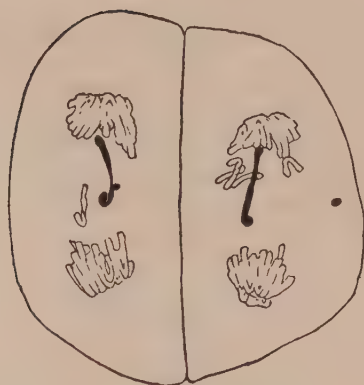


FIG. 14. Second anaphase bridges and fragments in *T. Sphaerococcum* \times 'blue' wheat

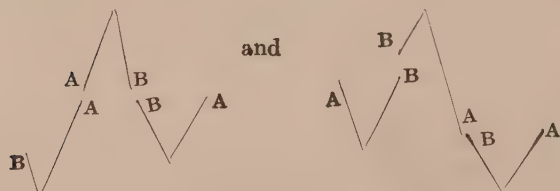
2. *T. sphaerococcum* \times B. Two associations of three (6) are found in the same cell. The average number of univalents is 8.4 (Table V). The number of chiasmata per potential bivalent and that for each individual cell are 29.6 and 29.60 respectively. The coefficient of terminalization is 0.68. The coefficient of bridgidity evidenced by the occurrence of bridges (Fig. 14) is 0.00135.

DISCUSSION

A. Configurations

1. *Tetraploid hybrids*. The 17 multiple configurations found in 20 cells of *T. durum* \times B. W. are exclusively associations of four. More than 76 per cent of the latter are ring-shaped and the remainder are chains of four, either in the shape of N or of an open ring.

In *T. turgidum* \times B. W. the multivalents are most frequently associations of three. The one which fails to pair is always the one of the two with the median centromere. For the association of three only two combinations are common, i.e.



In *T. pyramidalis* D. H. W. the multiple configuration is always an association of four. Among the cells studied one is devoid of an association of four instead of which bivalents are present.

2. *Pentaploid hybrids*. In *T. vulgare* \times B. W. only two cells possess 14 (II) + 7 (I) are found. Among the six cells possessing multiple configurations four show apparent reduction of chiasma frequency as well as increase in the number of univalents; showing that the formation of multiple configurations

must have something to do with the decrease in the number of chiasma frequency and the increase in the number of univalents. These will be dealt with in later paragraphs.

In *T. sphaerococcum* × B. W. none of the five cells examined show more than 13 bivalents. The combinations of the configurations are mostly 13 (II) + 1 (I), 1 (IV) + 12 (II) + 7 (I), 1 (III) + 11 (II) + 10 (I), 2 (III) + 11 (II) + 1 (I), and 1 (IV) + 11 (II) + 9 (I). The maximum number of associations of three is two and in the same cell the number of univalents is seven showing that there is intrahaploid pairing owing to external interchanges.

The formation of multiple configurations in the hybrids mentioned in this paper is all due to external interchange and this had been proved in the following ways:—

(a) Formation of bivalents in haploid plants of wheats—thus the number of bivalents was found to be one in *T. monococcum* [Kihara and Katayama, 1933; Chizaki, 1934], three in *T. durum* [Kihara, 1936]. In *T. vulgare* the number of bivalents was found to be one to two [Gaines and Aase, 1926], three [Yamamoto, 1936], four [Yamasaki, 1936] and nine [Krishnaswamy, 1939]. Krishnaswamy even found an association of three.

(b) Multiple configuration in pure species—thus in *T. turgidum*, Darlington 1931, 1] found an association of four.

(c) Intrahaploid pairing in intergeneric hybridization—in intergeneric hybrids a part of the bivalents is due to intrahaploid chromosome pairing in *tritium*. Bivalents were found in *T. turgidum* × *Haynaldia villosa* [Berg, 1934], and in the hybrids between *T. turgidum* and species of *Aegilops* [Percival, 1930].

T. durum showed bivalents on hybridization with *Ae. ventricosa* [Katayama 1931; Matsumoto, 1933] and with *Secale cereale* [Kagawa and Chizaki, 1934; Plotonikowa, 1930].

Bivalents were also found in *Ae. ovata* × *T. vulgare* [Kattermann, 1932], *T. vulgare* × *Secale cereale* [Aase, 1930] and *Ae. ovata* × *T. sphaerococcum* [Percival, 1930].

(d) Intra or intergenomic pairing in intragenetic hybridization—Thompson [1926] found no multiple configurations in *T. monococcum* × *T. turgidum*. In *T. durum* × *T. monococcum* only a trace of multiple association was found [Aase, 1930]. In *T. vulgare* × *T. monococcum* four to twelve bivalents were found [Kostoff, 1935]. The extra five bivalents besides the normal seven are due probably to pairing between chromosomes of *vulgare*. The formation of numerous extra bivalents in *T. vulgare* × *T. monococcum* together with the rare occurrence of multiple configurations in *T. durum* × *T. monococcum* evidently shows that there are interchanges of chromosome segments between chromosomes of the same or of different genomes.

Contrary to the above findings, some previous investigators found no multiple configurations in *T. turgidum* × *T. vulgare* [Watkins, 1924], *T. sphaerococcum* × *T. turgidum* [Vakar, 1932] and *T. turgidum* × *T. durum* [Osano, 1935]. The occurrence of multiple configurations in *T. vulgare* × B. W., *T. sphaerococcum* × B. W., *T. durum* × B. W., and *T. turgidum* × B. W. shows that the external interchanges of the chromosomes of 'blue' wheat are long enough for autosyndesis to take place.

The percentage of ring is much higher in *T. durum* \times B. W. than that obtained in tomato [Upcott, 1935]. In all the hybrids, the multiple configurations are almost exclusively rings and chains. Interstitial chiasmata are formed only between homologous members (Figs. 2, 3 and 5). Multiple configurations other than ring or chain require more than one chiasma on the same arm [Upcott, 1935], the failure of the latter indicates that the external interchanges between non-homologous chromosomes form terminal chiasmata only [Upcott, 1937, 2] and thus the duplications are not long enough for the chiasmata to be formed.

Morphologically the multiple configuration in *T. turgidum* \times B. W. is similar to one of the two in *T. durum* \times B. W. (Figs. 7 and 8). The constant similarity in the morphology of the multiple configurations of both hybrids suggests that the two sets of four chromosomes may be the same. If this is the case, then *T. durum* has two more common segments on those four chromosomes which form another association of four (Fig. 2) with 'blue' wheat than *T. turgidum* with 'blue' wheat.

The frequent failure of the association of four chromosomes in *T. turgidum* \times B. W. is due probably to the lower homology between the non-homologous members, as for the two types of configurations obtained there is only a four of chance that the fourth one can pair with its homologue.

The above mentioned shows that the homology of the homologous chromosomes is higher in *T. turgidum* and 'blue' wheat than in *T. durum* and 'blue' wheat; the homology between the non-homologous members is inversely true for the two hybrids.

In *T. pyramidale* \times dwarf hill-wheat, the interchanges are not only short, terminal ones but are limited to two pairs of chromosomes. All other chromosomes show normal pairing, except the inverted regions.

B. Interference of pairing between configurations

Among the 10 cells mentioned in *T. vulgare* \times B. W., there are four cells wherein association of more than two chromosomes is correlated with an increase in the number of univalents as well as a decrease in the frequency of chiasmata. It seems likely that chiasma formation between more than two chromosomes interferes with the chiasma formation of the other members. It is due probably to interference of pairing during the zygotene stage [Darlington, 1937]. This coincides with the finding in *Matthiola incana* by Armstrong and Huskins [1934] that the increase of multiple associations resulting from translocations and duplications is correlated with the decrease in normal pairing. The frequencies of the multiple configurations, the univalents and the chiasmata for individual cells are listed in Table VI.

The relation between the multiple configuration and the total chiasma frequency for every individual cell is detected by computing the coefficient of contingency between the two factors mentioned. The 'C' value and its standard error are 0.992 ± 0.005 , showing that multiple configurations are negatively correlated with the frequency of chiasmata. The reduction in the chiasma frequency is undoubtedly due to interference in normal pairing which is a consequence of the formation of multiple configurations.

TABLE VI

Distribution of multiple configurations, univalents and chiasmata

Cells	1	2	3	4	5	6	7	8	9	10
multiple config.	1	0	0	1	0	2	1	0	1	1
univalents	5	11	7	10	7	10	13	7	12	6
chiasmata	36	30	32	26	30	27	25	37	29	32

Ribbonds [1937] in *Lilium* \times *testaceum* found that there was no relation between the frequency of univalents and the chiasma frequency of the reining bivalents. The writers' results show that the frequency of univalents negatively correlated with the chiasma frequency. The value of 'rank relation' and its standard error amounting to -0.755 ± 0.136 show that frequency of univalents is a net index of the failure of pairing which is a sequence of interference.

Thus the interference caused by the formation of multiple configurations be detected by either the chiasma frequency or by the frequency of the univalents. The frequency of univalents is positively correlated with the frequency of multiple configurations and the chiasma frequency is negatively related with the latter.

Interference between configurations other than the multiple configurations [Rother, 1936] cannot be detected because the separate bivalents cannot be distinguished.

Structural changes

1. *Inversions.* Bridges of the first division are due to crossing-over in the inverted region which results in an acentric fragment and a dicentric chromatid. Bridges in the second division are due to crossing-over in the inversion loop which results in a loop and an acentric fragment in the first division and thus a bridge and fragment in the second division [Darlington, 1937].

(a) Number of inversion : The occurrence of two single bridges in one cell in *T. pyramidale* \times B. W. indicates that there are two chromosomes possessing inversion. In all the other hybrids except *T. vulgare* \times 'blue' wheat only one chromosome possesses such an inversion.

(b) Size of inversion : The size of the acentric fragment equals the sum of the length of the inversion and the portion distal to the inversion [Upcott, 1937, 2]. The small size of the fragment (Figs. 11, 13 and 14) shows that the portion of the inversion can hardly be large.

(c) Position of inversion : In *T. durum* \times B. W. the bridge is quite thick and the arms of the bridge are only a little bit shorter than the thick portion of the bridge (Fig. 9). Thus the container of the inversion and its homologue

probably have sub-median centromeres and the inversion is near to the end of the longer arm [Richardson, 1936; Upcott, 1937, 2]. This is supported by the small size of the fragment. The chromosomes involved in *T. durum* × *B. W.* and *T. vulgare* × *B. W.* are probably similar members (Figs. 9 and 13). Another bridge (Fig. 12) from a different cell of *T. vulgare* × *B. W.* shows a different morphology. The chromosomes probably have median centromeres. If this is so, then *T. vulgare* × *B. W.* is heterozygous for at least two inversions. In *T. sphaerococcum* × *B. W.* the inverted segment is near to the end of the longer arm of the chromosomes possessing subterminal centromeres (Fig. 14). It seems likely that the container of the inversion and its homologue are a different pair of chromosomes in comparison with *T. durum* × *B. W.* and *T. vulgare* × *B. W.* The two bridges are due to the two loops and two fragments of the first division resulting from a chiasma proximal to the inversion, which is disparate with respect to complementary chiasmata in the inversion.

In *T. pyramidale* × dwarf hill-wheat the inverted segment is on the shorter arm, because the bridge is thin and the arms are, on the contrary, long and thick (Fig. 10) [Richardson, 1936]. The other chromosome possessing an inversion has sub-median centromere (Fig. 11).

The striking thing to which attention should be paid is that so far as our material is concerned no bridge formation occurs in *T. turgidum* × *B. W.* This logically follows that *T. durum* but not *T. turgidum* differs from 'blue' wheat by an inversion. Here again, a higher degree of homology is revealed between chromosomes of *T. turgidum* and 'blue' wheat than those of *T. durum* and 'blue' wheat.

2. *External interchanges.* The details are mentioned under 'A. Configurations'.

3. *Undefined structural changes.* The undefined structural changes result in the reduction of the frequency of chiasmata [Darlington, 1937]. The general difference of the chromosomes is shown in Tables II to V. The higher degree of homology between the homologous chromosomes of *T. turgidum* and 'blue' wheat not only gives 0.45 more bivalents (in comparison with *durum* × *B. W.*) but also shows a higher frequency of chiasmata for every individual bivalent, every potential bivalent and a higher average total frequency of chiasmata per cell. In *T. turgidum* × *B. W.* 76.6 per cent of the total chiasmata is terminal as compared with 90.5 per cent in *T. durum* × *B. W.*, in other words, 23.4 per cent in the former and 9.5 per cent in the latter are interstitial. Since a higher frequency of chiasmata, especially that of interstitial ones, is always positively associated with the degree of affinity, therefore, we have another evidence that phylogenetically 'blue' wheat is more closely related to *T. turgidum* than to *T. durum*.

The frequency of chiasmata in *T. pyramidale* × *D. H. W.* is nearly as high as that in *T. turgidum* × *B. W.* (Table III). It shows not only a similar distribution, but also a very close mean as compared with that in *T. turgidum* × *B. W.* A comparison of the bivalents possessing different number of chiasmata is shown in Fig. 15, and the distribution of total chiasmata in individual cells is listed in Table VII.

Both the frequency of chiasmata per potential bivalent and that per cell in *T. vulgare* × *B. W.* are similar to those in *T. sphaerococcum* × *B. W.*; but

the latter the coefficient of terminalization is somewhat lower. The distribution of total chiasmata for individual cells of the pentaploid hybrids is stated in Table VII.

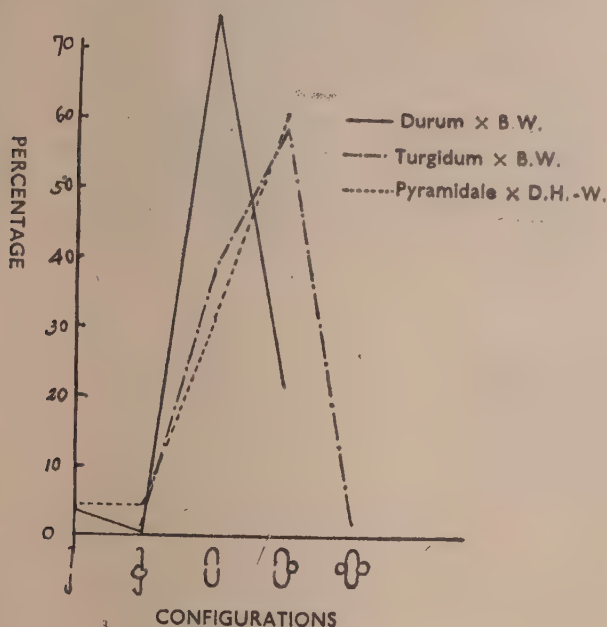


FIG. 15. Showing the comparison of the bivalents possessing different number of chiasmata

TABLE VII

Distribution of total chiasmata for individual cells

Chiasmata	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	No. of cells observed	Mean & S. D. m
<i>Durum</i> x B. W.		1		3	4	4	4	2		1	1						20	30.15 ± 0.460
<i>Turgidum</i> x B. W.							1	2	3	3		1	2	3		1	16	35.00 ± 0.647
<i>Pyramidale</i> x B. W.						2	2				2	1	2	1			10	34.40 ± 0.812
<i>Blonde</i> x B. W.	1	1	1		1	2		2				1	1				10	30.40 ± 0.194
<i>Macrococcum</i> x B. W.		1		1		1		2									5	29.60 ± 0.043

Anaphase division

1. *Behaviours of univalents.* The univalents are distributed at random and usually divide in the first and lag in the second division. They form

supernumerary nuclei during the tetrad stage, if they are not included in daughter nuclei.

In *T. pyramidale* \times D. H. W. the univalent laggard may fail to divide (Fig. 16) in the first division. There is no doubt of its being a true univalent [Upcott, 1937, 1]. Its failure to divide is due probably to a delay in moving on to the equator [Darlington, 1937].

2. Fragmentation of univalents has been observed in the second anaphase of *T. sphaerococcum* \times B. W. (Figs. 17, 18 and 19), due probably to the division of centromeres described by Upcott [1937, 1] and Darlington [1939]. Mis-division takes place in both divisions. A four-to-none type of division (Fig. 19) has been observed. They more frequently show normal division in the first anaphase and mis-divide in the second anaphase (Figs. 19 and 21). Mis-division of the centromere is due to the double structure of the centromere [Darlington, 1939, 1940].



FIG. 16. Failure of division of a univalent lagged in anaphase I of *T. pyramidale* \times 'blue' wheat

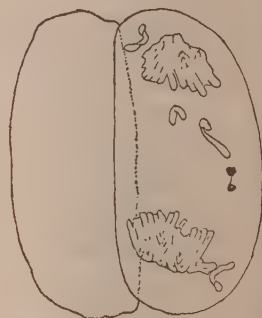


FIG. 17. Mis-division of a univalent in the second division of *T. sphaerococcum* \times 'blue' wheat

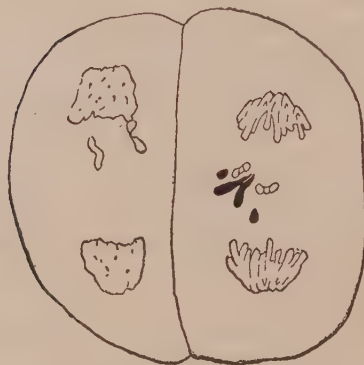


FIG. 18. Mis-division of univalent. A 4-0 type of division takes place in the first division leaving two centric and two acentric arms. Besides, there is, probably, a free centromere

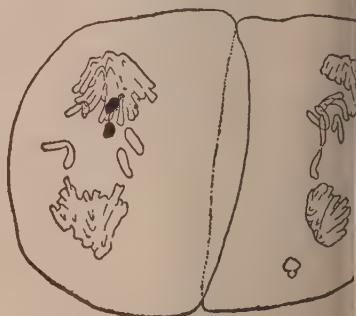


FIG. 19. A univalent mis-dividing in the second division

Non-disjunction of bivalents

2. Non-disjunction of bivalents has frequently been observed in *T. durum* × B.W. (Fig. 20). This is due to the presence of the interstitial chiasmata to which there is probably a change of homology which makes complete terminalization impossible. This is similar to the finding of Darlington [1931,2] in *Oenothera* and that of Philp and Huskins [1931] in *Matthiola*.

Formation of unbalanced gametes

During the second metaphase of *T. pyramidale* × B. W. a plate showing $n = 16$ has been observed (Fig. 21). This is due, most probably, to non-disjunction of a multiple association of which the centromeres lie indifferently with one another.

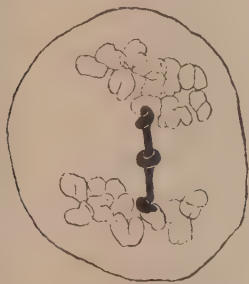


FIG. 20. Non-disjoined bivalent in *T. durum* × 'blue' wheat



FIG. 21. Metaphase II in *T. pyramidale* × D. H. W., showing $n = 16$

SUMMARY

Judging from the multiple configurations, the occurrence of inversions and comparison of the general frequency of chiasmata as well as the average number of bivalents, 'blue' wheat is phylogenetically nearer to *T. turgidum* than to *T. durum*.

The occurrence of only one association of four and the high frequency of chiasmata together with the absence of wax as well as the dwarf height of the plant which rarely exceeds 3 ft. reveal that dwarf hill-wheat might be related to *T. pyramidale*.

The pentaploid hybrids between *T. vulgare*, *T. sphaerococcum* and 'blue' wheat show two multiple configurations. The numbers of univalents are 8.8 for *T. vulgare* × B. W. and 8.4 for *T. sphaerococcum* × B. W.

Bridges are observed in all the hybrids except *T. turgidum* × 'blue' wheat. The formation of multiple configuration is correlated with the decrease in normal pairing in *T. vulgare* × 'blue' wheat.

Non-disjunction of bivalent and formation of unbalanced gametes are observed in the tetraploid hybrids.

Fragmentation of univalents is observed in *T. sphaerococcum* × 'blue' wheat.

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STUDIES IN THE PERIODIC PARTIAL FAILURES OF THE PUNJAB-AMERICAN COTTONS IN THE PUNJAB

AMELIORATION OF *TIRAK* ON SOILS WITH SALINE SUBSOILS
(SANDY LOAMS)

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(With Plates XXVII and XXVIII)

One of the previous contributions [Dastur, 1941] the ameliorative effect on the opening and yields of the application of the sulphate of ammonia to cotton plants that developed *tirak* on account of a deficiency of nitrogen on light sandy soils was described. When the sulphate of ammonia was applied to such light sandy soils the premature yellowing and shedding of leaves did not occur in the crop, the opening of bolls improved and the value of *kapas* was greatly increased. A rapid method of spotting the deficiency of nitrogen in the crop called the 'tannin test' was also dealt with. It was equally important to develop remedial measures for amelioration of cotton soils with saline subsoil and intensive studies were undertaken on this aspect of the problem.

It has already been pointed out [Dastur and Sucha Singh, 1942] that the effect of 'physiological disorder' that sets in plants growing on saline subsoils is different from that which occurs in plants on light sandy soils. In the former case the remedial measure for such soils was simple after such soils were treated. In the case of soils which have high salinity in the subsoil, the method of removing or counteracting salinity at a depth of 3-4 ft. is rather difficult and complicated. The probable remedial measures for cotton subsoils can be classified into three groups; (1) There are 'known' measures which would counteract the toxic effects of sodium salts. Under this category may be mentioned the application of gypsum or any other calcium salt which would antagonize the toxic effects of free soluble sodium and which by a process of base exchange may replace the exchangeable sodium with calcium in the clay complex. The toxicity of sodium salts is known to depend on the physical texture of the soil. Higher percentages of

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clay or presence of organic matter in the soil may detoxicate the sodium. Application of organic manure like the farmyard manure or the green in like berseem or additions of silt (a substitute for clay) were then regarded as possible remedies. The use of inorganic fertilizers like nitrogen superphosphates and potash was also considered as they might not be available to the plants from such soils. (2) Salinity in the subsoil may be washed down from the feeding zones of the roots by flooding such fields. This may be combined with application of gypsum. (3) Measures by which occurrence of water deficit in the cotton plants at the reproductive stage may be either removed or avoided. This group would naturally include the applications of water to increase the amount of available water by keeping non-saline upper surface moist and the reduction of the total leaf area of plants by cutting down their vegetative growth.

The applications of these remedial measures become specially difficult on account of great heterogeneity that is prevalent in the Punjab soils. intermingling of normal soils, saline soils and light sandy soils in the field, presents difficulties for applications of substances to counteract the effect of sodium salts or of washing them down to lower layers by flooding. Salinity occurs in such an irregular manner that such measures would have to be applied even where they were not required. As for instance flooding would wash down the salts from the feeding zones of the roots in those parts of the field where salinity is present in the subsoil but it would also leach the important nutrients from normal non-saline areas, especially from the sandy portions, and render them infertile. It is, therefore, necessary that the treatment that is either applied to the soil to counteract or to remove salinity in the subsoil must be such as to ameliorate the soil conditions in the saline areas while it does not at the same time in any way adversely affect the soil conditions or the growth of plants in non-saline parts. If this point is not borne in mind the removal of one evil may be accompanied by the creation of another.

Another difficulty is that the treatments for counteracting the toxic effect of salinity must penetrate to a depth of 3 ft. or more in order to be effective. That would render their use impracticable as well as uneconomical.

INVESTIGATION

The occurrence of *tirak* was noticed in the cotton season of 1931 in a field at the Lyallpur Agricultural Farm. The soil conditions and the behaviour of the plants growing on this field were studied, side by side. Positions of the normal and *tirak* patches were carefully marked and the soil conditions under normal and *tirak* crop were investigated. It was established that *tirak* had developed in regions where the subsoils were saline. This field measured 138 ft. \times 288 ft. was, therefore, particularly selected for the trial of remedial measures of the type discussed above, in the cotton season of 1931.

The ameliorative treatments were: (1) flooding, (2) gypsum + flooding, (3) inorganic fertilizers (sulphate of ammonia + superphosphate) + flooding, (4) gypsum + flooding + inorganic fertilizers and (5) farmyard manure followed by flooding. The sixth treatment was control.

With the exception of inorganic fertilizers, the other applications were far in advance of sowings. Gypsum was added in two doses of 500 lb. per acre in December, 1937 and January, 1938 followed by flooding time. Flooding was also started from December. Each time the land in *wallar* it was ploughed up. Flooding was done three times before sowing for sowing was given. Farmyard manure was applied at the rate of tons per acre. The sulphate of ammonia was applied at the rate of 10 lb. N per acre and the superphosphate at the rate of 150 lb. P_2O_5 before sowing. Thus an attempt was made to include all the remedial measures of the two categories.

The remedial measure of the third category was also tried. The idea was to cut down the leaf area by reducing the vegetative growth and the natural way to accomplish this was to defer sowings by one month as compared with the normal sowings. As a preliminary trial the inclusion of sowing dates as treatments was, therefore, considered desirable. The field was divided into four blocks. Each block consisted of two main plots for two sowing date treatments. The main plots were subdivided into six sub-plots to which were assigned at random the six treatments enumerated above. It was, therefore, a split plot design with eight main plots consisting of sub-plots of $1/80$ acre each.

Thus it would be normally expected that the most precise information would be obtained on the six sub-plot treatments while the information that might be obtained on sowing dates will be less precise as they were allocated to main plots. Unsown interstrips were kept in between sub-plots to avoid shading and seepage effects.

Sowings were done on 5 and 6 May, and 7 and 8 June in the early and late sown plots respectively by dibbling as the small size of the plots did not permit the use of a hand drill. Eight seeds per hole were sown at $2\frac{1}{2}$ ft. \times $2\frac{1}{2}$ ft. distance. Each hole was equidistant from the six surrounding holes (equilateral system). On account of a heavy shower of rain on 12 June, received soon after germination, the late sown plots had to be resown on 15/16 June. Thus the two sowing dates under trial were 5/6 May and 15/16 June. The plants were finally thinned to two per hole. The early-sown crop received in all eight waterings, while the late sown received six waterings. With the exception of the first two waterings to the early-sown crop, subsequent irrigations were given on the same day to both the sowings. The last date of watering for both the sowings was 18 October.

The crop in each plot was under close observation throughout the season. The first abnormality noticed in the condition of the plants under each treatment was noticed in the May-sown cotton plants began to show drooping of leaves. The drooping of the leaves in the May-sown plants was noticed irrespective of the six ameliorative treatments in all plots where the soil was saline while drooping of leaves was not noticed in any one of the plots which were sown in June. Thus one of the symptoms of 'disease' was completely absent in the June sown plots indicating that reduction in leaf area prevented the development of the water deficit that occurred on such plots. The June sown plants were much smaller in size and consequently had less foliage than the May-sown crop.

Drooping of leaves was followed by excessive defoliation of the sown plants while shedding was much less in the June-sown crop under similar conditions as compared with the early-sown crop at the same stage of morphological development of the plant. The leaves of the two crops presented striking differences in colour and appearance at fruiting; the leaves remained green and fresh in the June-sown plants and dull and blackish in the May-sown plants.

At the time of flowering and fruiting the two sowings exhibited important and distinctive features that deserve mention. A delay in sowing of about 40 days shifted forth the onset of flowering by about 12 days only. The flowers were mostly confined to the tips of the branches in the May-sown crop while in the June-sown the lowermost nodes of the main stem did not directly produce the flowers. The branches which were located high up on the main stem and the secondary branches. In the June-sown plants the sympodia arose directly from the nodes on the main axis and the flowers did not, therefore, appear aggregated at the tips as they were widely spaced on them. The growth of the flowers and branches was more vigorous in the late-sown than in the early-sown crop so the successive flowers were separated by longer internodes in the June-sown (Plate XXVII, figs. 1 & 2).

The most reliable criterion to judge the quality of opening of the boll is the weight of seed cotton per boll. The bolls that are badly opened (as in the bolls of *tirak* plants) contain immature seeds and poor lint and, therefore, the weight of seed cotton per boll falls. Contrariwise, the maturity of the boll would be reflected in raising the weight of *kapas* of a boll and will be indicative of good opening. It was, therefore, undertaken to determine the effect of different treatments on the weight of seed cotton per boll. Two uniform pair of plants each were tagged at random in each sub-plot for this purpose. The number of opened bolls from these samples and weight of seed cotton produced by them were recorded before each picking. The yield of seed cotton in the experimental area in each sub-plot was then weighed and recorded.

Nodal counts and height measurements of the individual plants for boll weight in each sub-plot were taken when pickings were over. After the number of sticks and their weight were determined per plot. These determinations would provide information on the growth made by the plants under two sowing dates and the six sub-plot treatments. With the records of the weight of seed cotton produced by 100 gm. of stem dry matter, it can be computed to get an idea of the efficiency of the cotton plants for the production of seed cotton under different treatments.

The data collected were subjected to statistical analysis. The results of the analyses of variances are given below (Table I). A summarized record of the nature and magnitude of the effect of different treatments is given in Table II. The differences between the two dates of sowing were highly significant in all determinations despite inadequate replication. The sub-plot treatments did not differ significantly among themselves. The interaction of sowing dates with sub-plot treatments was non-significant, indicating no differential behaviour of time of sowing with the different treatments.



FIG. 1. Early-sown (5 May) 4F P.-A. cotton plant (leaves removed) showing that flowers and bolls are borne near the tips of main stem and branches



FIG. 2. Late-sown (16 June) 4F P.-A. cotton plant (leaves removed) showing that flowers and bolls are not aggregated at the top, but are borne at the lower as well as the upper nodes of the branches



FIG. 1. Badly opened bolls with lower leaves shed of the May-sown crop on soils which are saline in the subsoil



FIG. 2. Well-opened normal bolls of the late-sown crop on soils which are saline in the sub

Due to	Yield in gm.		No. of bolls		Weight of seed cotton per boll		Height in cm.		Stem weight per plant gm.		Seed cotton per 100 gm. stem weight	
	D.F.	Mean square	F	Mean square	F	Mean square	Mean square	F	Mean square	F	Mean square	F
Blocks	3	8634455.0	24.886**			0.3787	502.1	..	9150.7	..	217.82	..
Dates	1	143036172.6	393.411**	145782.1	148.96**	4.1251	8234.1	15.81*	252735.2	53.77**	23306.86	230.16**
Error (a)	3	359016.3	..	981.3	..	0.1467	520.7	..	4693.9	..	105.43	..
Treatments	5	630280.7	..	774.64	..	0.0194	42.1	..	1365.6	..	66.33	..
Dates \times Treatments	5	513032.6	..	1535.60	..	0.0680	200.9	..	2303.6	..	61.12	..
Error (b)	30	1112926.1	..	2128.99	..	0.1374	127.8	..	1261.9	..	29.22	..

*Significant at 5 per cent level of significance
 **Significant at 1 per cent level of significance

TABLE II
Treatment effects on the vegetative and the reproductive characters
Experiment I.

	Control	Gypsum	Flood- ing	NP	Gypsum + NP	F. Y. M.	Means for sow- ing dates	Difference	
<i>Average yields in lb. per plot</i>									
May-sown (D1)	7.5	8.3	9.2	8.2	7.8	7.5	8.1	6.0**	2
June-sown (D2)	15.3	14.2	14.7	16.4	15.4	13.6	14.1	...	
Mean	11.4	11.2	12.0	12.3	11.6	10.5	
<i>Average No. of bolls per hole</i>									
May-sown (D1)	25.7	28.9	31.4	25.7	31.2	26.2	28.2	15.6**	
June-sown (D2)	42.6	43.5	42.1	47.7	44.0	42.7	43.8	...	
Mean	34.2	36.2	36.8	36.7	37.6	34.4	
† Sampling error per plot = 10.9 per cent of the mean									
<i>Average weight of seed cotton per boll in gm.</i>									
May-sown (D1)	1.89	1.95	1.95	1.78	1.86	1.87	1.88	0.42**	
June-sown (D2)	2.35	2.20	2.32	2.42	2.29	2.21	2.30	...	
Mean	2.12	2.07	2.13	2.10	2.07	2.04	
‡ Sampling error per plot = 5.9 per cent of the mean									
<i>Average height in cm. per plant</i>									
May-sown (D1)	101.6	109.6	109.4	103.4	100.6	104.8	104.8	18.5*	14
June-sown (D2)	85.4	84.3	83.1	89.9	92.3	82.7	86.3	...	
Mean	93.5	96.9	96.3	96.6	96.6	96.5	
‡ Sampling error per plot = 3.4 per cent of the mean									
<i>Average weight of stem per hole</i>									
May-sown (D1)	283.3	341.5	334.5	353.8	311.0	294.5	319.3	141.7**	1
June-sown (D2)	181.3	152.5	162.0	180.0	189.0	173.0	174.6	...	
Mean	232.3	247.0	248.0	266.9	255.0	233.8	
<i>Average seed cotton per 100 gm. of stems</i>									
May-sown (D1)	19	16	19	16	17	19	17.6	42.9**	
June-sown (D2)	60	67	64	65	51	56	60.5	...	
Mean	39.5	41.5	41.5	40.5	34	37.5	

*Significant at 5 per cent level of significance

**Significant at 1 per cent level of significance

***C. D. at 5 per cent level of significance

† Sampling errors per plot for various determinations were of an order so as to justify that the samples were fairly representative of the entire plots. The conclusions drawn were, therefore, applicable to the entire plots.

‡ Low sampling error for height indicates that this character is less variable than boll number or weight of cotton per boll

The performance of deferred sowing was superior to May sowing in of yields, boll numbers and the weight of seed cotton per boll. As mentioned the late-sown plants did not exhibit the *tirak* symptoms drooping of leaves and their premature shedding. As the opening of bolls in the late-sown crop was significantly superior to opening of bolls in May-sown crop, the maturity of seeds in the bolls in the former case greater than that of the latter. Apparently the mean value 1.88 gm

of seed cotton per boll is high for a *tirak* crop. But that was due to reasons; firstly, the field was fallow during the previous year and received of preliminary tillage and, secondly, the soil was not uniformly saline in subsoil over the entire field. Salinity in the subsoil was present in places only, while non-saline areas were scattered about at random in certain portions having either low salinity or no salinity in the subsoil. The yield of seed cotton in the plots where the soil was saline had gone down to 10 gm. per boll but that was not the case with the late-sown plants under similar soil conditions. This difference was reflected in the average weight of seed cotton per boll in the June-sown crop.

Evidently the late sowing had reduced the vegetative growth as the yield per plant and stem dry matter per plant were significantly lower in the June-sown plants than those attained by the May-sown.

The late sowing had definitely ameliorated *tirak* and the conception of increasing the leaf area of the plant so that water deficit in the crop may not be so severe was found to hold good.

The efficiency for production of seed cotton in the late-sown crop appeared to be significantly higher than that of the early-sown crop. The proportion of seed cotton produced per unit dry matter of stems was much higher in the late-sown than in early-sown plants. This is important as what was required was more of seed cotton rather than of the vegetative growth.

The ameliorative effects of similar treatments for counteracting the serious effects of salinity in the subsoils were again studied in the same field in the 1939-40 cotton season. Sowing date was omitted from the experiment and the effect on *tirak* was studied in a separate experiment to be described later. This experiment was repeated with some changes in the nature of the treatments. The plots under flooding in the previous year were treated with silt at the rate of 80 tons per acre. The plots receiving farmyard manure in the previous year were green manured at the rate of 10 tons per acre. The treatment gypsum and inorganic fertilizers of 1938-39 was substituted with green manuring supplemented by the same inorganic fertilizers, the latter being applied as a split application in two equal doses (half at sowing and half at flowering, total quantities added being the same as in the previous experiment). The remaining three treatments were the same, viz. gypsum, green manure and phosphorous, and control and they were allotted to the same plots. On account of the omission of sowing dates eight replicates of six treatments could then be provided. Thus the layout was a simple randomized block arrangement.

The drooping of the leaves began to be noticed by the beginning of September in all plots where the subsoil was saline irrespective of the treatment given. As the season advanced it was evident that the drooping occurred on larger areas of the field and was acuter in form in this season than was found in 1938-39. The *tirak* had spread to parts of the field which had not been affected in the previous season. The same was the case when the bolls opened. The drooping was more pronounced and widespread in this season than in the previous one. Thus the *tirak* was more intense than what it was in the previous year. This aggravation in *tirak* as already explained in the previous section [Dastur and Samant, 1942] was due to a continuous spell of very dry and warm weather that prevailed in September and October.

The water deficit in the crop was accentuated by weather conditions caused *tirak* symptoms to develop on soils which had low salinity in the surface.

The number of bolls per plant, the weight of seed cotton per boll yields and the weight of stem dry matter were recorded as before. Statistical analyses revealed the treatment variance to be of the same order as the variance in case of boll numbers, weight of seed cotton per boll yields. As the 'z' test indicated that the treatments were not significant it was not considered worth while to proceed further with the statistical analysis. The mean values for yields per plot, for the number of bolls per plant and the weights of seed cotton per boll are given below (Table III). The general level of yields was very low because of severe *tirak*.

TABLE III
Treatment effects on yield, boll number and boll weight
Experiment II

	Control	Gypsum	Silt	Green manure	NP	Green manure + NP	Mean
Yields in lb. per plot	2.01	2.92	2.35	1.71	2.55	2.71	2.38
Number of bolls per sq. yd.	40.8	46.5	52.0	38.0	50.6	51.0	46.5
Weight of seed cotton in gm. per boll	0.69	0.87	0.76	0.65	0.83	0.81	0.77

No ameliorative effect of any one of the treatments was found either in the opening of the bolls or the yields as was the case in the previous year.

The magnitude of differences between the yields and weights of seed cotton of the May-sown crop in the two seasons 1938-39 and 1939-40 was as great as can be seen by comparison of Table III with Table II.

A 2^4 factorial experiment was designed in all combinations of (D_1, D_2) (S_1, S_2) (W_1, W_2) (N) where D_1 = crop sown on 14 May, D_2 = crop sown on 21 June, S_1 = close spacing 2 ft. \times 1½ ft., S_2 = wide spacing 2½ ft. \times 1½ ft., W_1 = normal watering, W_2 = heavy watering in September-October, N = 50 lb. of nitrogen in the form of sulphate of ammonia applied in August. The layout (shown in the Appendix) was a 8×8 quasi-factorial design. All the four second order interactions were partially confounded with the soil differences of columns and the third order interaction was completely confounded with the soil differences of the rows. Thus an attempt was made to minimize the effect of soil heterogeneity by eliminating two sources of systematic soil variations. The size of each sub-plot was 1/113 acre and sowing.

Observations on the crop under different treatments showed that the drooping of the leaves occurred from middle of September in the May-sown crop in plots with saline subsoil. This was confirmed by analyses of soil samples. The drooping symptoms were greatly reduced in such plots which received heavy waterings. No such drooping of leaves was noticed in the plots which were sown in June (D_2). The drooping of leaves was followed by shrivelling.

the first week of October. The bolls in such plots cracked and opened early.

It may be recalled here that the spread and intensity of *tirak* were also greater in this field in this season of 1939-40 as compared with the previous season, i.e. 1937-38 when cotton was grown in the same field.

In a couple of plots the June-sown crop was as badly opened as the late-sown, showing no ameliorative effect of late sowings. Such plots were found to contain a greater percentage of sand than others in the first 2 ft. of the soil and small amounts of alkalinity present within 2 ft. of the surface were found to be toxic to the roots. The early and late-sown plots did not make normal growth. They were stunted in growth and produced a large number of badly opened bolls. Except for such plots or portions of such plots the late-sown crop did not show *tirak*. Statistical analysis has, however, been carried out without rejecting such plots.

The number of bolls per plant and the weight of seed cotton per boll were determined from duplicate random samples. Each sampling unit comprised of six holes (two plants per hole) in S_1 and three holes in S_2 sub-plots. The yield, the height and the weight of stems were taken as before. The data for weight of seed per boll, the yields and the dry matter of stems

TABLE IV

Analyses of variances(2⁴ confounded design in 8 × 8 quasi-Latin square, 1939-40)

Due to	D. F.	Weight of <i>kapas</i> per boll		Yield of <i>kapas</i>		Dry weight of sticks	
		Mean square	F	Mean square	F	Mean square	F
Replication	7	0.1392		26.6547		822.18	
Blocks	7	0.2262		28.3832		2280.62	
Factor A	1	1.1250	8.66**	2.3831		27121.97	296.17**
Factor B	1	0.6992	5.38*	191.1652	10.60**	285.19	3.11
Factor C	1	0.0325		2.7183		8.34	
Factor D	1	0.2965		191.5802	10.63**	1706.72	18.63**
Factor E	1	0.5408	4.16*	165.7335	9.193**	365.29	3.99
Factor F	1	0.0205		10.7339		1.79	
Factor G	1	0.0914		33.1920		87.19	
Factor H	1	0.0004		4.0251		21.27	
Factor I	1	0.0800		5.6228		36.75	
Factor J	1	0.2945		76.0166	4.22*	57.19	
Factor K	1	0.0117		0.8374		121.60	
Factor L	1	0.4320		56.8981		410.67	
Factor M	1	0.0026		11.0784		24.80	
Factor N	1	0.3927		3.2751		6.31	
Factor O	35	0.1299		18.0277		91.57	

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

were analysed by the method appropriate to the design. The detail analyses of variances are given in Table IV. The significant main effects and interactions with respective standard errors are shown in Table V under appropriate subheadings.

TABLE V
Summary tables showing main effects and significant interactions
Experiment III

Treatment	Average weight of bolls per plant in gms.	Difference with S. E.	Yield in pounds per acre	Difference with S. E.	Weight of seeds in pounds per acre	Difference with S. E.
D ₁	1.47	**	13.82	0.39 ± 1.06	22.02	**
D ₂	1.29	0.18 ± 0.64	13.46		64.09	-41.17
W ₁	1.46	*	15.95	**	45.82	
W ₂	1.31	0.15 ± 0.64	11.89	0.40 ± 1.06	41.4	4.22
N	1.36	-0.06 ± 0.64	13.41	-0.17 ± 1.06	43.15	-0.72
O	1.45		15.82		48.87	
S ₁	1.48	-0.01 ± 0.64	15.95	**	48.67	**
S ₂	1.33		11.89	0.46 ± 1.06	38.34	10.33

	D ₂	D ₁	Difference (0.001)		D ₂	D ₁	Difference (0.001)		D ₂	D ₁	Difference (0.001)	
D.W. interaction	W ₂	1.42	1.45	0.06	W ₁	16.77	16.94	-2.88	W ₁	68.6	22.65	—
	W ₂	1.35	1.48	0.13**	W ₂	10.66	10.70	-3.81*	W ₂	59.6	23.2	—
	Difference	0.27**	.02		Difference	6.68**	0.24		Difference	9.0*	—55	
		±0.001				±1.50				±3.38		

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

The three treatments which were tried for amelioration of *tirak* were (1) deferred sowings, (2) heavy watering and (3) application of nitrogen. The last was included only as a precautionary measure though it has been already shown that deficiency of nitrogen is not the cause of bad opening on these soils. A study of Table V will show that both the ameliorative measures deferred sowings and heavy waterings, significantly increased the weight of seed cotton per boll indicating better opening of bolls, i.e. less of *tirak*. The interaction of sowing-dates with waterings (D.W) was significant showing that opening of bolls in May-sown crop was greatly improved by heavy watering while the late-sown crop showed no further improvement in opening by application of water, as the improvement in opening obtained by deferred sowings was of a high magnitude. The plants under late sowings did not require extra water as no disturbance in the water balance occurred in the late-sown crop. This was indicated by absence of drooping of leaves in late-sowing. Nitrogen, as expected, had no effect on the weight of seed cotton per boll. Thus the two remedial measures late sowing and heavy watering at the fruiting stage proved effective in improving opening of bolls, the former to a greater extent than the latter.

The generalized effect of watering on yields was significant at 1 per cent level of significance and the interaction sowing date \times watering was also significant. The early-sown crop profited considerably by extra application of water while no benefit accrued to the late-sown. The increase in yield due to heavy watering in the former was 6.6 md. per acre while the increase was practically nil in the latter. Thus extra watering had helped the early-sown crop in increasing both the yields and the weight of cotton boll while no similar advantage from heavy watering was derived by the late-sown in any case.

The generalized effect of spacing on yields was positive and significant indicating that the yield under close spacing was higher than that under wide spacing. Though the interaction of dates and spacing (D. S) did not prove significant, the following data (Table VIa) would show that the late-sown crop benefited more by close spacing than the early-sown. The effect of spacing derives its significance from the significant effect of close spacing in D_2 only, the effect of close spacing under D_1 alone being non-significant.

TABLE VI

Interaction of sowing date with spacing on yield and the effect of sowing dates on waterings on the efficiency of the plant

(a) Average yield in maunds per acre			(b) Seed cotton per 100 gm. of stem weight (efficiency)			Mean
	D1	D2		D1	D2	
Early-sown	14.44	16.27	W1	17.21	60.9	39.05
Late-sown	12.42	11.36	W2	25.24	64.59	44.92
	+2.02	+4.91**	Mean	21.23	62.74	

* Significant at 1 per cent level of significance

As the early-sown plants were benefited more by watering than the late-sown plants and as wide spacing had acted against the late sowings, the generalized response to sowing dates was small and non-significant. As the interaction of sowing dates and watering was significant, no importance attaches to the generalized effects as such.

The interaction of spacing with nitrogen on yield was found to be significant. This was due to an anomalous decrease under wide spacing in the absence of nitrogen. It is possible that some of the plots with wide spacing and no nitrogen came on soils which had high salinity in the subsoil.

The effects of sowing date on growth characters confirmed the conclusion already reached that late-sown plants remain stunted and produce comparatively less dry matter. Heavy waterings increased the weight of sticks significantly on early-sown only, thus bringing out a significant interaction between sowing date and watering.

The efficiency of plants for production of seed cotton was worked out in this experiment also. The effect of heavy watering and late sowing on the proportion of seed cotton produced per 100 gm. of stem dry matter is given in Table VI (b). Obviously the late sowing influences the efficiency of plant seed cotton production. Watering is also effective but cannot compare with late sowing in this respect.

The ameliorative effects of deferred sowings on *tirak* occurring in soil with saline subsoil was further determined in another experiment arranged at the Risalewala Seed Farm, Lyallpur in the cotton season of 1940-41. The experiments discussed in the foregoing pages were conducted with 4F Punjab American cotton variety only. It was, therefore, necessary to extend the studies by introducing in such experiments a number of *desi* and American varieties. Such a study would disclose not only the relative resistance of different American strains to *tirak*, if any, but also their suitability for adoption for late sowing.

Accordingly 18 varieties, 15 Americans and three *desis* were included in the experiment. Out of the entire lot under trial, there were six commercial varieties, four Americans and two *Desis* while the rest were newly evolved promising strains which were kindly supplied by the Cotton Research Botanist, Lyallpur.

The layout conformed to randomized blocks design with sub-plot arrangement. The entire area consisted of six blocks (320 ft. \times 119 ft.) of five main plots each (80 ft. \times 119 ft.). Four sowing-date treatments were distributed at random to the main plots within each block. Two rows, 119 ft. length for each of the 18 strains were accommodated in each main plot. The position of varieties within each plot was perfectly randomized. Non-experimental belts were cut out on all sides at pickings to avoid border effect to the main-plot treatments. There was no scope for the provision and subsequent rejection of edge rows to eliminate border effect on variety comparisons. The marked reduction in the standard error of the variety comparison by split plot arrangement, however, dispels the possibility of a considerable border effect influencing the sub-plot treatments. The experimental sub-plot measured 100 ft. 10 in. \times 4 ft. (1/108 acre).

Sowings were done on 8 May, 23 May, 7 June and 23 June in D_1 , D_2 , D_3 , D_4 plots respectively. At the time of thinning, the plants were spaced closer and closer as cotton was sown successively later. The spacings adopted for the different sowings were: $D_1 = 2$ ft. \times 2 ft., $D_2 = 2$ ft. \times 1½ ft., $D_3 = 2$ ft. \times 1 ft., $D_4 = 2$ ft. \times 9 in. The first and the second sowings received all eight and seven waterings respectively while each of the June-sown sowings was given five irrigations, i.e. three irrigations less than the first sowing. Except for such additional earlier irrigations to the May-sown plots, it was so arranged that subsequently all sowings were watered on the same date.

Yield records were maintained throughout the picking season. Different varieties have to be picked every week and thus it was not convenient to record their boll weights in this experiment. Boll weight determination from sampled plants were, therefore, confined to 15 American varieties of early sowing.

A study of analyses of variances (Table VII) revealed that there were significant variations among the four sowing dates in case of yield as well as that of *kapas* per boll. The varietal comparisons were also highly significant on both of them. The interaction 'dates' 'varieties' attained significance in yield only.

TABLE VII

Analyses of variances (sub-plot basis)

(Varietal and sowing-date experiment at Risalewala Seed Farm 1940-41)

Due to	Boll weight			Yield of <i>kapas</i>		
	D. F.	Mean square	F	D. F.	Mean square	F
Replications	5	2.3096	3.396*	5	48.5325	2.419
Sowing dates	3	12.60266	18.531**	3	116.2191	5.791**
Plots (a)	15	0.6801	..	15	20.0695	
Sub-plots	23		
Replications	14	1.1544	13.346**	17	140.4478	70.2239**
Sowing dates	42	0.0876		51	5.7876	2.573**
Plots (b)	280	0.0865		340	2.2490	

Components of the sum of squares due to dates of sowing

vs. D_3+	1	27.1590	39.934**	1	135.2625	6.74*
vs. D_1+	1	8.1421	11.972**	1	203.7615	10.153**
vs. D_2+	1	2.5067		1	9.6332	

*Significant at 5 per cent level of significance

**Significant at 1 per cent level of significance

The total sum of squares attributable to the three degrees of freedom for sowing dates was further split up by regarding them as quality treatments and contributions of orthogonal components were tested against the error (a). The last two sowings were found to differ from the first two significantly and the differences were relatively much more pronounced on yield than on yield. The behaviour of the central sowings was also significantly different from the two extreme sowings. It is, therefore, necessary to refer to the detailed Table VIII to grasp the nature and magnitude of effects of the variables under study.

There was a progressive rise in the weight of *kapas* per boll with delay in sowing upto the third sowing beyond which there was little effect. Thus a well marked optimum towards the June sowings was clearly brought out with respect to the opening of bolls. The magnitude of increase was sufficiently high. The sowing dates stood in the order D_3, D_4, D_2, D_1 according to merit and this order remained virtually the same in the different varieties taken individually. This accounted for the non-significant interaction between the two factors. Improvement in opening in all the varieties brought about to the same extent by delay in sowing. The mean boll weight of the varieties showed significant variation among themselves. This indicates inherent varietal differences in boll sizes of the different strains. Boll weight is a composite measure of the all-round development of seedling of a given variety but higher boll weights in certain varieties in comparison to others, do not necessarily imply a corresponding reduction in percentage immaturity of seeds. Varieties having large and fuzzy bolls may suffer to the same extent by *tirak* and yet may possess markedly higher seed weights due to more of non-essential parts, as compared with non-*tirak* strains under similar soil conditions.

TABLE VIII

Results of the experiment at Risalewala Seed Farm, 1940-41

	Average weight of <i>kapas</i> per boll					Mean yield in lb. per sub-plot (1/108)			
	D_1	D_2	D_3	D_4	Mean ± 0.06	D_1	D_2	D_3	D_4
L88 . . .	1.37	1.56	2.11	2.08	1.78	3.83	5.08	6.66	6.80
4F . . .	1.16	1.44	1.80	1.47	1.49	2.91	4.38	7.25	4.34
289F/43 . .	1.19	1.74	2.18	1.68	1.70	2.98	4.20	5.11	4.61
289F/K25 . .	1.30	2.00	2.22	2.00	1.88	3.97	5.78	5.23	4.32
L88 early . .	1.55	1.84	2.32	2.22	1.98	4.64	9.63	11.27	9.70
289F/124 . .	1.42	1.84	2.28	2.26	1.95	5.26	6.98	7.22	6.77
289F/126 . .	1.70	2.03	2.72	2.48	2.23	5.07	6.50	8.14	7.05
289F/127 . .	1.43	1.90	2.17	2.26	1.94	4.95	6.65	4.16	4.38
289F/144 . .	1.40	1.94	2.19	2.08	1.90	4.77	5.90	6.67	5.26
289F/155 . .	0.86	1.40	1.60	1.64	1.37	2.37	4.91	4.84	4.26
289F/156 . .	1.21	1.86	2.37	2.12	1.89	4.66	7.61	8.40	6.06
289F/157 . .	1.24	1.73	2.14	1.98	1.77	5.41	6.90	6.45	5.53
289F/158 . .	1.36	1.87	2.35	2.30	1.97	4.23	7.21	7.33	6.40
289F/159 . .	1.38	2.14	2.03	2.03	1.91	5.57	8.26	6.26	5.47
289F/186 . .	1.12	1.41	1.87	1.79	1.55	3.45	4.77	6.22	5.69
DU17 . . .						10.0	8.92	11.71	10.22
Mo1 39 . . .						12.09	10.74	15.14	13.32
Sang 119 . .						9.24	11.01	12.03	10.58
Mean . . .	1.31	1.78	2.16	2.03		5.29	6.96	7.78	6.71

S. E. of the body of the table (interactions and varietal effects only) ± 0.087
 ± 0.12

± 0.431
 ± 0.612

The optimal value for yields was obtained in the third sowing after which there was a tendency for falling off in the effectiveness of further delay in sowing. This was attributable to a diminution in the boll number per unit area caused by a reduction in growth and also by some jassid attack in the late sowing in the susceptible varieties. Even then the mean yields of the third sowing were higher than those of the first sowing and compared favourably with those obtained from the second sowing. The varieties susceptible to jassids attained an earlier optimum in relation to sowing date than others resistant to them. This explains partly the significant interaction.

CONCLUSIONS

The results discussed above clearly indicate that *tirak* occurring on soils which have a saline subsoil can be ameliorated by either reducing the water requirements of the crop by means of deferred sowings or by applications of extra water from the beginning of the flowering phase so that upper non-aqueous layers may adequately meet the demand of the crop. The first remedy of deferred sowings is to be preferred to the second as the former enables the crop to meet its own demands for water without external aid. The former is also a more practical remedy than the latter as the water supply is usually limited. There is considerable observational and experimental evidence to support the view that the late sown crop is better adapted to its topographic and climatic environment than the early-sown (May-sown) crop. The late-sown crop shows no symptoms of water starvation and consequently is able to mature its crop of bolls under saline conditions of the soil or unfavourable conditions of weather or both. A late-sown plant is thus in physiological equilibrium with its environment and is able to stand the vagaries of weather which many a time is dry and warm during the fruiting period. It is also a more efficient organism than a May-sown plant. It produces more seed cotton in proportion to its size than what an early-sown plant does. The latter exhausts itself in the vegetative growth and by the time bolls are formed, it has already reached a stage of senescence. A slightly higher temperature than normal for a brief spell of three weeks or so is sufficient to upset its metabolic processes on such saline subsoils, for the plant has lost its capacity for adjusting itself to such changes in its environment. A particular advantage is also gained by the sowing of the cotton crop in May even on non-saline soils as the crop exhibits a kind of photoperiodism. The flowering phase does not set in early in an early-sown and if it does, such bolls do not develop into bolls and are generally shed. The onset of flowering is not proportionately delayed as the sowings are delayed; a shift in the date of sowing does not materially influence the main flowering period which occurs in the month of September. Early sowings will be advantageous only when the flowering period is also considerably prolonged. A longer flowering period would enable the crop to mature a larger number of bolls than what they do. But as the matters stand the early-sown crop becomes unbalanced with a long vegetative phase and a short reproductive phase. This lack of balance between the two phases results in a low efficiency in production of seed cotton.

The June-sown crop, however, suffers from a disadvantage as compared with the early-sown crop. As the vegetative phase is shortened the bearing points are reduced resulting in a reduced number of bolls. But this disadvantage can be counteracted by closer spacing of plants, i.e. by increasing the number of plants per acre. This measure will make up for the decrease in bearing on the late-sown plant and the crop will at the same time be less susceptible to *tirak* on saline subsoils and will produce better quality of lint.

A large number of experiments laid out on zemindars' fields have substantiated the conclusions discussed above and these results will be discussed in another contribution.

The ameliorative measures for counteracting the toxic effects of salinity in the subsoil such as the use of gypsum, silt and green manuring have not proved effective and *tirak* occurred irrespective of these treatments. These measures would also be beyond the means of zemindars, even if they were successful. There would also be difficulties of their application to the right place as *tirak* also occurs on soils which are not saline in the subsoil [Dast and Samant, 1942].

The attempt to leach down the salts by flooding to deeper layers of soil has also not proved successful. Applications of nitrogenous fertilizers and superphosphates produced no ameliorative effect on *tirak* on such soils.

SUMMARY

Tirak or bad opening of bolls in the Punjab-American cottons on soils with saline subsoils is mainly caused by a disturbance in the water balance of the plant. A water deficit arises in the plants towards the fruiting stage which is the most critical period of plants' life and becomes more and more pronounced with the march of time. Salinity in the subsoil renders the absorption of water difficult and the plants succumb to the physiological drought. Three types of ameliorative measures were tried for counteracting the toxic effect of salinity: (1) applications of gypsum, silt, farmyard manure and green manures, (2) washing down of the salts from the feeding zones of the roots by flooding of such lands and (3) efforts for preventing the development of a water deficit by means of cutting down the vegetative growth (e.g. by late sowings) or by giving extra applications of water at the fruiting stage.

Replicated field experiments were conducted to study the effects of the three types of ameliorative measures during the cotton seasons of 1938-39, 1939-40 and 1940-41 on such lands where subsoil salinity was known to exist and where *tirak* had previously occurred.

Of the three types of ameliorative measures, the two measures of the third group, viz. deferred sowings (June sowings) and extra applications of water from the flowering stage proved successful in remedying *tirak* while the measures of the first two types failed to produce any effect.

Deferred sowings did not show drooping of leaves in October as was the case with the May-sown crop on such soils. There was also no premature defoliation. The opening of bolls (weight of seed cotton per boll) and yields were significantly better in the June-sown crop than those of the May-sown crop. Similarly, heavy watering from fruiting stage lessened the

ably and increased the yields in comparison to normal waterings. Any watering had no effect on the opening or the yield of June-sown crop. The latter did not stand in need of extra water and was not profited by it. Sowing was found to be superior to heavy watering in effect on *tirak*.

June sowings produced less number of bolls per plant than May sowings on account of a reduction in the vegetative growth in the former. This was a disadvantage in late sowings but it was successfully counteracted by increasing number of plants per acre. This could be done by adopting closer spacing of plants.

The experiment laid out in 1940-41 was a varietal-cum-sowing-date trial. There were four sowing dates equally spaced at fortnightly intervals commencing from the second week of May with 15 American varieties and three Indian varieties.

The results of boll size (weight of seed cotton per boll) showed that opening of bolls improved as the sowings were delayed. The opening of bolls in the two June sowings was significantly better than that of the May sowings. The improvement in opening was universal to all the varieties included for study. Similarly, the mean yields of the former were significantly higher than those of the latter. Varieties differed in their adaptability to late sowing. The strains resistant to jassids were in general better suited to the June sowing while those susceptible to them had a well defined optimum towards the central sowings (end of May to second week of June). The first sowing gave lower yields under all varieties taken individually.

The crop when sown in June is in a physiological equilibrium with its environment on soils with saline subsoil. It does not suffer from water starvation on such soils while the May-sown crop does. The production of seed cotton in proportion to plant size (dry weight) is much higher in late-sown as compared with that of the early-sown. The disadvantage of reduction in yield which usually accompanies this practice can be counteracted by increasing the number of plants per acre by reducing the distance between rows and adopting closer spacing from plant to plant within the rows.

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APPENDIX

Layout plan with treatments and yields in maunds per acre of the 2⁴ factorial design on 4-F cotton in sq. 27 D₁, 1939-40
(8 × 8 quasi-Latin square)

Column No. 1	D1 W1 S1 8.15	D1 W1 S2 9.88	D2 NW1 S2 13.34	D2 NW1 S1 25.89	D1 NW2 S1 12.81	D1 NW2 S2 20.68	D2 W2 S2 15.19	D2 W2 S1 21.01
Column No. 2	D1 NW2 S1 21.68	D2 W1 S1 17.22	D2 NW2 S1 17.72	D1 W1 S1 15.04	D1 NW1 S2 7.57	D2 W2 S2 10.71	D2 NW1 S2 14.80	D1 W2 S2 21.22
Column No. 3	D1 W2 S2 20.99	D2 NW1 S2 10.78	D1 W1 S2 12.69	D2 NW2 S2 10.16	D2 W2 S1 15.23	D1 NW1 S1 9.57	D2 W1 S1 14.76	D1 NW2 S1 17.66
Column No. 4	D1 NW1 S2 8.52	D1 NW2 S2 13.62	D2 W2 S2 10.25	D2 W1 S2 10.08	D1 W1 S1 22.66	D2 NW2 S1 12.70	D1 W2 S1 12.24	D2 NW1 S1 18.54
Column No. 5	D2 W1 S2 11.59	D1 NW1 S1 3.23	D2 W1 S1 10.03	D1 NW1 S2 6.77	D1 W2 S2 17.70	D1 W2 S1 10.78	D2 NW2 S1 18.14	D2 NW2 S2 9.86
Column No. 6	D2 W2 S1 16.34	D2 NW2 S1 13.89	D1 NW2 S2 6.80	D1 W2 S2 20.14	D2 W1 S2 11.44	D2 NW1 S2 5.01	D1 NW1 S1 6.01	D1 W1 S1 7.21
Column No. 7	D2 NW2 S2 10.24	D2 W2 S2 11.18	D1 W2 S1 14.99	D1 NW2 S1 28.37	D2 NW1 S1 10.45	D2 W1 S1 10.80	D1 W1 S2 8.08	D1 NW1 S2 7.45
Column No. 8	D2 NW1 S1 14.40	D1 W2 S1 17.50	D1 NW1 S1 23.19	D2 W2 S1 17.40	D2 NW2 S2 12.70	D1 W1 S2 5.96	D1 NW2 S2 11.75	D2 W1 S2 13.62

RECOVERY OF WHITE SUGAR FROM THE PUNJAB AND THE UNITED PROVINCES CANES

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THE investigation described in this paper was carried out during the two cane seasons 1937-38 and 1938-1939, in order to throw light on the marked differences in the percentage recovery of sugar from canes imported to the Punjab from the United Provinces for the then existing Sonapat Sugar Factory and the corresponding canes grown locally. The Punjab Sugar Corporation reported that the recovery for local Sonapat canes was 7.7 per cent as against 10.18 per cent for corresponding canes imported from Amroha or elsewhere in the United Provinces. A systematic analytical survey of the canes grown in the two provinces, combined with investigations of the soils on which the canes were actually grown, was accordingly carried out in order to throw light on the differences in percentage recovery recorded. The canes in particular areas in the Punjab and the United Provinces in which this investigation was carried out in 1937-38 unfortunately experienced an exceptionally bad attack of pyrilla, which to a considerable degree rendered the results less conclusive than was desired. Valuable data were, however, collected during the first year's survey but owing to the abnormal conditions it was postponed in the following year, i.e. 1938-39, as it is obvious that in order to obtain reliable comparative data on such a matter as this the data must be collected during a season when normal conditions prevail. It is, however, almost impossible to find any extensive area during any season, where what might be called 'absolutely normal conditions' do prevail. For example during the second year of the investigation although pyrilla was practically absent, the canes in both the provinces were subjected to drought of greater severity than during the first year.

The United Provinces grow more than half the total cane produced in India, the chief tracts being the Meerut, the Rohilkhand division in the west, and the Gorakhpur division in the extreme east.

For the investigation under report eight localities were selected, four from the eastern United Provinces, namely Meerut, Bisokhar, Salimpur and Amroha, and four from the south-eastern Punjab, namely Karnal, Tharu, Rohat and Bahli. In the 1938-39 survey, the districts in the Punjab were the same but in the United Provinces, Billari village in the Moradabad district and Shahdaspur were added and Bisokhar omitted.

Owing to the above-mentioned attack of pyrilla and the removal of the Sonapat Sugar Factory to Amroha there were only 2,895 acres under

sugarcane in the 1938-39 season in the Sonapat tehsil, as against 11,557 in 1937-38 season. Also, very little of this cane of the second year was for the manufacture of *gur* or sugar but was chiefly employed as fodder account of famine. Similar conditions prevailed in the United Provinces where a number of factories did not work for more than two months.

CLIMATE

The western part of the United Provinces receives a higher rainfall than the south-eastern Punjab, the monsoon usually setting in about the middle of June from which date monsoon conditions prevail until about the end of September. Furthermore, the eastern portion of the area in the U. P. report usually gets a considerably higher rainfall than the western part as shown from the data in Table I. The rainfall, however, during the monsoon prior to the first year's investigation was below normal in all the localities investigated and this factor occurring at a period when the cane is at a stage of maximum growth caused the crop to be stunted both in the United Provinces and the Punjab. This undoubtedly constituted one of the predisposing causes to the severe attack of pyrilla during the ensuing cane season, during which there were occasional frosts which, however, were not severe enough to do much harm. The major trouble was pyrilla.

TABLE I

Rainfall in inches during the last five years at the localities under survey

Locality	Years					Average
	1934	1935	1936	1937	1938	
Meerut (Western United Provinces)	30.25	21.52	23.46	17.88	9.79	
Muzaffarnagar (for Salimpur) (Eastern United Provinces.)	36.24	31.43	41.58	32.51	26.32	
Amroha (Eastern United Provinces)	22.99	39.39	50.93	26.99	31.95	
Shahjahanpur (Eastern United Provinces)	38.04	25.75	70.23	22.78	39.43	
Sonapat (South-eastern Punjab)	17.35	17.25	23.85	19.92	9.32	
Karnal (South-eastern Punjab.)	31.74	43.15	34.26	25.09	18.60	

We have already mentioned how the trouble from pyrilla was reflected to a certain extent in a smaller acreage under cane in 1938-39 than in 1937-38. It is also how difficult it is to find, what may be called, a normal season. In the first year the rainfall in the previous monsoon was below normal and was complicated with an attack of pyrilla and in the second year although there was freedom from pyrilla yet the rainfall in the previous monsoon was lower than in the first year both in the Punjab and the United Provinces, particularly at Sonapat. As a result of this deficient rainfall during the period when

cane crop most needed it, the crop in both the provinces was stunted and consequently matured earlier. In the 1938-39 cane season, there was no frost and consequently quality was maintained throughout the short crushing season.

SOILS AND GENERAL AGRICULTURAL PRACTICES

The nature of the soils from that part of the United Provinces investigated and of the south-eastern Punjab varies from sandy to medium loams tending to somewhat heavier types in the subsoil. On the whole, however, the analytical data from these soils and agricultural experience show them to be eminently suited, in both provinces, to the production of cane. Average analytical data computed from separate investigations in a number of localities in both the provinces are given below :—

Percentage on air-dried soils

Depth	Total nitrogen	Organic matter	Exchangeable calcium	Available P_2O_5	Maximum water-holding capacity	Water-soluble salts	Clay	pH
United Provinces—								
1st foot	0.0553	0.650	0.096	0.055	37.3	0.117	13.4	6.58
2nd foot	0.0448	0.426	0.144	0.042	37.0	0.102	21.2	6.72
3rd foot	0.0392	0.376	0.187	0.032	38.5	0.090	24.8	6.81
Punjab—								
1st foot	0.0740	0.900	0.158	0.035	40.7	0.200	19.4	6.95
2nd foot	0.0548	0.706	0.182	0.030	40.6	0.170	25.7	6.78
3rd foot	0.0496	0.583	0.186	0.031	40.6	0.178	27.6	6.62

An interesting point brought out in the survey was that the soils of the south-eastern Punjab, are comparatively heavier and contain a greater amount of exchangeable calcium than the soils from the western United Provinces. There is not much difference in the reaction of the two soils although the United Provinces soils have slightly lower pH values. The differences in the value of the two sets of soils for sugarcane production appear to lie in the differences in the amounts of water-soluble salts and exchangeable calcium in the soils of the two provinces. In the Punjab the soils contain a considerable concentration of water-soluble salts which accumulate near the surface, but in the United Provinces, where rainfall and humidity are both greater and where the soil is lighter, the percentage of water-soluble salts is much less. It is perhaps this fact more than any other which may influence the ash content of the sugarcane juice and subsequent percentage recovery of sugar in the factory, and which may consequently form an explanation of the alleged difference in the value of two sets of canes in sugar production. As shown by Lander and

Ramji Narain [1936], it is the greater amount of ash in the juice from Punjab canes rather than its slightly lower sucrose content as compared with the juice of the sugarcane from the United Provinces, which is responsible for the lower net rendiment value of the *gurs* made from the Punjab canes. There are certain other factors also which appear to have a definite bearing both on the quantitative and qualitative production of cane in the United Provinces and the Punjab. The most important of these appear to be climatic in regard to the relative degree of rainfall, and the variations in certain important aspects of agricultural procedure. In the United Provinces *barani* land usually produces a moderately good crop of cane which could not be produced on corresponding lands in the Punjab. Again, average well-cultivated soils in the Punjab usually receive far more natural manure than the average corresponding United Provinces soils, which accounts for the fact that the former are generally richer in nitrogen and organic matter than the latter. There are exceptions, however, and in some parts of the United Provinces, intensive manuring produces soils richer in nitrogen and organic matter than is found in average Punjab soils. Again, the cost of cultivation owing to generally more economic conditions is lower in the United Provinces than in the Punjab, which reason alone is a more extensively grown crop in the former province. Of the total cultivated area in the localities surveyed, about 20 per cent is under cane in the United Provinces, against 10 per cent in the Punjab. On the other hand, the standard of cultivation and the amount of manure used are, as a rule, higher in the Punjab, so that land irrigated by well or canal water in the Punjab generally produces more cane per acre than corresponding land in the United Provinces.

PESTS

As already mentioned, the 1937-38 cane crop in both the western United Provinces and south-eastern Punjab was severely damaged by pyrilla. The high-yielding varieties, such as Co 312, suffered most, and the more luxuriant and succulent the leaves the heavier was the attack. The natural result was a juice of inferior quality with a diminished sucrose content and so high a glucose content that *gur* could not be prepared from it. As a typical example the following table shows the deterioration of Co 312 as a result of this attack.

Co 312 (Karnal) attacked by pyrilla

Date of analysis	Percentage on sugar cane						Purity of juice
	Juice	Sucrose	Glucose	Total sugars	Total solids	Glucose ratio	
9 Dec. 1937	71.8	3.2	1.65	4.9	6.1	51.1	5
27 Dec. 1937	75.6	4.3	2.08	6.4	7.8	48.4	5
12 Jan. 1938	74.9	4.0	1.81	5.8	7.3	45.2	5
24 Jan. 1938	74.6	3.7	1.82	5.5	6.6	49.2	5
8 Feb. 1938	75.2	4.8	1.79	6.6	7.6	37.3	6

During the following season the crop was free from pyrilla and the cane ripened normally as shown below :—

Co 312 (Karnal) free from pyrilla (1938-39)

Date of analysis	Juice	Sucrose	Glucose	Total sugars	Total solids	Glucose ratio	Purity coefficient
Dec. 1938	69.3	9.6	0.73	10.3	11.6	7.6	82.8
Dec. 1938	67.9	8.9	0.29	9.2	10.7	3.0	83.2
Dec. 1939	67.6	11.2	0.26	11.5	12.6	0.9	88.9
Feb. 1939	71.6	11.3	0.10	11.4	12.8	1.2	88.3
Feb. 1939	66.7	10.1	0.14	10.2	12.3	1.4	82.1
Feb. 1939	72.1	11.0	0.14	11.1	12.5	0.8	88.0

PLAN OF WORK DURING THE SURVEYS

In considering the data from different localities surveyed it may be mentioned that all the varieties at each place were analysed six times during each season from the beginning of December till the first week of March. It is not proposed to give all the analytical data collected each year, but only the average composition of different varieties, together with figures for the glucose ratio, purity coefficient and saline coefficient for the maturity periods. The figures for the yield of stripped cane, total solids and sucrose per acre, have also been recorded. The soils from the fields from which the canes were analysed were sampled at a number of places to a depth of 3 ft. and corresponding 1 ft. samples from all the bores were mixed together, so that three composite samples were obtained from each locality for analysis.

LOCALITIES SURVEYED

The United Provinces

Government Agricultural Farm, Meerut

The soil of this farm is an average loam but becomes somewhat heavier below the first foot. The average composition of the soils of this farm is given below :—

Percentage on air-dried soil

	Total nitrogen	Organic matter	Water-soluble salts	pH	Exchangeable calcium	Available P_2O_5	Clay	Silt	Sand
1 ft.	0.0680	0.970	0.180	7.81	0.120	0.046	15.1	25.4	59.44
1 ft. to 2 ft.	0.0476	0.421	0.160	8.34	0.136	0.030	24.9	31.4	43.70
2 ft. to 3 ft.	0.0476	0.405	0.180	8.54	0.124	..	27.7	32.8	39.48

The soil is alkaline in reaction, possesses a moderate amount of exchangeable calcium and a moderately high percentage of water-soluble salts.

Four varieties of canes were examined, viz. Co 244, Co 312, Co 313 and Co 311, the analytical data from which are given in Table II.

TABLE II
Analytical data of the varieties of cane grown at the Government Agricultural Farm, Meerut
 (Average during the ripening period)

Particulars	Percentage on cane						Glucose ratio	Purity coefficient	Saline coefficient	Yield per acre in maunds			Ripening period	
	Juice	Sucrose	Glucose	Total sugars	Total solids	Non-sugars				Ash				
Co 244 : 1937-38	71.7	9.7	0.59	10.5	11.4	0.9	0.409	6.1	83.1	24.0	586.3	56.8	66.8	5/1 to 14/2
1938-39
Co 312 : 1937-38	72.3	8.8	0.80	9.6	10.8	1.2	0.405	9.1	81.4	22.0	920.6	81.0	99.4	31/1 to 14/2
1938-39	69.2	9.6	0.37	10.0	11.3	1.3	0.399	4.1	85.3	25.7	940.0	90.2	106.2	18/12 to 20/2
Co 313 : 1937-38	68.1	10.1	0.25	10.4	11.4	1.0	0.548	2.4	88.6	18.5	547.9	55.3	62.4	17/1 to 14/2
1938-39	77.2	10.7	0.18	10.9	12.3	1.4	0.482	2.0	86.2	24.1	659.9	70.6	81.2	6/12 to 20/2
Co 331 : 1937-38	69.3	9.0	0.50	9.5	10.8	1.3	0.503	5.5	85.3	18.0	607.1	54.6	65.5	31/1 to 14/2
1938-39	67.7	9.8	0.45	10.3	11.4	1.1	0.451	3.6	85.8	22.7	865.0	84.7	98.6	18/12 to 20/2
1937-38	70.4	9.4	0.56	10.0	11.1	1.1	0.466	5.8	85.6	21.1	666.0	61.9	73.5	..

Co 312 gave the highest outturn of 920·64 maunds per acre in 1937-38, and 940 maunds in the following season, but its maturity was somewhat delayed owing to the heavy manuring practised at the farm, and a larger number of irrigations than is usually given in the United Provinces.

The most important factors to be kept in mind in connection with the specific object of this survey are the ash content of the juice and the saline coefficient, the latter being the ratio between sucrose and ash. The canes from this farm showed the highest ash content and the lowest saline coefficient of any examined in the United Provinces, and were accordingly poorer in quality. It may be noted that in the 1937-38 season, yields from all varieties were lower than in 1938-39, probably owing to the attack of pyrilla in the former season, which was more severe at this place than at any other surveyed in the United Provinces.

Bisokhar

Bisokhar is one of the villages situated in the new sugar development area of the United Provinces and its soils are good sandy loams as their average composition given below shows :—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1 foot	0·0580	0·840	6·67	0·118	0·032	0·080	13·6	16·8	69·6
2½ foot	0·0420	0·474	7·22	0·134	0·028	0·100	19·1	16·5	64·4
3½ foot	0·0336	0·484	6·86	0·154	0·028	0·160	22·9	18·9	58·2

These soils contain a smaller percentage of water-soluble salts than those of the Meerut farm, whilst the organic matter and total nitrogen content is about the same.

Four chief varieties were examined at Bisokhar, viz. Co 244, Co 312, Co 313 and Co 331 (Table III). The last two had received a dressing of a mixture of ammonium sulphate and castor cake at the rate of five maunds per acre, which may have been responsible for the high ash content of the juice. It is interesting to note that the ash content of Co 244 and Co 312, which were manured here, was much less than that of the corresponding manured cane of the Meerut farm. This suggests that manuring may be one of the causes responsible for an increase in the amount of mineral matter in the juice of cane. The purity coefficient figures of the manured Co 313 and the unmanured Co 244, both of which are early-ripening varieties, indicate that manuring delays ripening—an observation which is also borne out by a consideration of the analytical data for Co 313 at Amroha which received no manures.

TABLE III
Analytical data of the varieties of cane grown at village Bisohar (1937-38)

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 244 .	68.1	11.1	0.53	11.6	12.4	0.8	0.302	4.9	89.5	39.4	350.0	38.4	43.4	3/12 to 16/2
Co 312 .	71.0	9.2	0.78	10.0	11.1	1.1	0.237	8.5	85.3	38.9	550.5	50.7	55.1	16/2
Co 313 .	70.3	10.3	0.36	10.7	12.1	1.4	0.647	3.5	85.1	16.3	640.5	66.0	77.5	17/12 to 16/2
Co 331 .	70.4	9.1	0.66	9.8	11.9	1.1	0.497	6.1	85.0	19.1	650.0	59.1	72.1	19/1 to 16/2
Average .	70.0	9.9	0.58	10.5	11.9	1.4	0.420	6.1	85.0	23.4	547.8	53.7	62.0	..

Simpur

This village situated about three miles from Muzaffarnagar has light loam soils similar to those of the Meerut farm, but they tend to become heavier below the first foot. The average composition of these soils is given below :—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0588	0.710	6.25	0.120	0.030	0.160	15.7	25.9	58.3
2nd foot	0.0532	0.307	6.25	0.166	0.046	0.160	25.7	28.2	46.1
3rd foot	0.0504	0.226	6.25	0.200	0.025	0.080	30.7	28.3	40.7

This soil is heavier than those of Bisokhar and Amroha and is adequately rich in nitrogen, organic matter and exchangeable calcium, and a uniform pH of 6.25 down to 3 ft. in depth was found, indicating its acidic nature.

The four varieties examined here, viz. Co 213, Co 244, Co 312 and Co 313 (see IV) were all ripe at the end of November or in the first week of December and their low ash content and high saline coefficients show them to be superior to the canes from Meerut and Bisokhar. The outturn, however, was poor. Co 312, for example, a heavy yielding cane gave only 425 maunds of sugar per acre. Compared with Amroha canes all varieties showed a low saline content but gave higher yields.

TABLE IV
Analytical data for the cane varieties grown at village Salimpur

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 244: 1937-38	69.9	11.4	0.58	12.0	13.0	1.1	0.349	5.0	87.2	33.7	375.0	42.7	48.7	8/12 to 20/2
1938-39	64.7	10.9	0.22	11.1	12.3	1.2	0.286	2.7	87.8	38.0	400.0	45.6	49.2	4/12 to 28/2
Co 213: 1937-38	67.5	10.7	0.39	11.1	12.3	1.2	0.478	3.7	87.0	29.9	350.0	37.4	43.0	8/12 to 20/2
1938-39	65.7	10.3	0.48	10.8	12.3	1.5	0.408	5.0	83.6	25.1	370.0	38.1	45.5	4/12 to 28/2
Co 313: 1937-38	70.0	11.4	0.39	11.8	12.8	1.0	0.421	3.4	89.1	27.9	410.0	46.1	52.5	8/12 to 20/2
1938-39	64.0	11.3	0.29	11.6	12.8	1.2	0.385	2.7	88.7	29.5	400.0	45.2	51.2	4/12 to 26/1
Co 312: 1937-38	70.8	9.7	0.90	10.6	11.7	1.1	0.340	9.3	82.9	29.5	425.0	41.2	49.7	8/12 to 20/2
Average: 1937-38	69.6	10.8	0.57	11.4	12.5	1.1	0.397	5.4	86.6	28.4	390.0	41.9	48.5	..
1938-39	64.8	10.8	0.33	11.1	12.4	1.3	0.359	3.4	86.6	30.9	390.0	42.3	48.6	..

Amroha

This village is situated in the Rohilkhand division and lies near the hills, for this reason the rainfall is considerable, and the climate more humid than that of Meerut and Bisokhar or the south-eastern districts of the Punjab. As may be seen from the table below, the soil is a sandy loam and, as judged from its content of organic matter, exchangeable calcium, water-soluble salts and total nitrogen, is the poorest of all the soils of the four localities investigated in the United Provinces. It is interesting to record that although the annual rainfall at Salimpur and Amroha is practically the same as each place, on other climatic aspects are similar, yet the Amroha soils contain the least amount of exchangeable calcium of any of those examined during this survey carried out in the United Provinces or the Punjab. The Amroha soils are very poor and this may partially account for their low content of exchangeable calcium—an observation which appears to hold good also for the soils of Ludhiana in the Punjab. The average composition of the Amroha soils is as follows:—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1 foot	0.0392	0.484	6.40	0.060	0.044	0.070	12.2	15.6	68.1
2 foot	0.0384	0.484	6.18	0.098	0.035	0.050	19.7	18.2	62.3
3 foot	0.0280	0.484	5.92	0.100	0.021	0.060	22.2	18.1	59.7

All the four varieties examined at Amroha (Table V), viz. Co 213, Co 312, Co 313 and Co 331 matured early and had a low ash content but a high purity coefficient, sucrose content and saline coefficients. Thus, the quality of the cane was the best of any in the United Provinces or the Punjab. For example, Co 313 had a sucrose content of 10.1 per cent, whilst the other three varieties had more than 11 per cent. It may be noted, however, that whereas the quality of cane produced was excellent, the outturn per acre was the lowest of the four. Co 213, which gave high yields at other places, gave only 256 maunds per acre at Amroha. From the factory point of view, quality is more important than quantity, but the cultivator's chief concern is quantity and in this respect interests conflict.

TABLE V
Analytical data of cane varieties grown at Amroha

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 213: 1937-38	68.1	11.2	0.35	11.6	12.6	1.0	0.364	3.1	88.9	33.0	256.0	28.7	32.2	12/2 to 22/2
1938-39	63.9	11.4	0.35	11.8	13.0	1.2	0.318	3.6	88.3	36.6	220.0	25.1	28.6	11/12 to 19/2
Co 312: 1937-38	71.7	11.2	0.54	11.7	12.6	0.9	0.219	4.8	88.9	60.1	350.0	39.2	44.1	6/1 to 22/2
1938-39	68.5	11.5	0.51	12.0	12.9	0.9	0.188	4.7	89.2	62.2	300.0	34.5	36.0	30/11 to 19/2
Co 313: 1937-38	66.6	11.9	0.34	12.3	13.1	0.8	0.243	2.9	90.8	52.3	300.0	35.7	39.3	12/2 to 22/2
1938-39	65.6	12.3	0.25	12.6	13.8	1.2	0.246	2.2	89.6	52.3	270.0	33.2	37.2	30/10 to 12/2
Co 331: 1937-38	69.0	10.1	0.78	10.9	11.8	0.9	0.213	7.7	85.6	49.0	341.7	34.5	40.3	12/12 to 22/2
1938-39	66.0	12.0	0.32	12.3	13.5	1.2	0.188	2.8	90.0	70.0	290.0	34.0	39.1	30/11 to 19/2
Average: 1937-38	68.9	11.1	0.50	11.6	12.5	0.9	0.260	5.1	88.6	42.7	312.0	34.5	39.0	..
1938-39	66.0	11.8	0.33	12.1	13.3	1.1	0.225	3.5	89.3	55.3	270.0	31.9	35.2	..

In this connection the following comparative figures for Meerut and Amroha are of interest :—

Maunds per acre

Locality	Season	Yield	Sucrose	Glucose	Ash	Available cane-sugar
Meerut	1937-38	666·0	62·0	3·84	3·10	49·6
	1938-39	822·0	81·8	2·46	3·74	66·2
Average	..	744·0	71·9	3·15	3·42	57·9
Amroha	1937-38	312·0	34·5	1·56	0·82	31·2
	1938-39	270·0	31·9	0·81	0·62	28·9
Average	..	291·0	33·2	1·19	0·72	30·1

It will be seen that, considering both the seasons, the amount of white sugar which could be obtained from an acre of sugarcane at Meerut was almost double that from the same area at Amroha, and from the point of view of the cultivator attempts to increase the yield deserve encouragement, but there appears to be a limiting point beyond which an increase in tonnage reflects in decrease in quality. The interests of the factory and the cultivator cannot be identical so long as the price of cane is fixed irrespective of quality. Another point noted in connection with the cane crop at Amroha was that the canes ripened from five to seven weeks earlier than those at Meerut. All factors which tend to increase the vegetative growth must delay ripening, and of these the amount of irrigation and manure applied are the most important. However, we find that the total amount of water, i.e. rain plus irrigation, applied to the cane crop at Amroha and at Meerut, was the same. As we have seen, the Meerut canes investigated received, in the 1937-38 season, five maunds of a mixture of castor cake and ammonium sulphate, and it is problematical whether this amount of manure could have delayed the ripening of cane by as long as five weeks. It is to be noted, however, that in the season 1938-39 the canes at Bilari were heavily manured, for in addition to the basic dressing of green manure with 300-400 maunds of sunn-hemp they received also two maunds of ammonium sulphate and 15 maunds of castor-cake per acre. These canes ripened about the end of January, whereas the Meerut canes which had received less manure than the above ripened about the middle of December, and the Amroha canes which were raised without any manure were ripe as early as the end of November. It would seem, therefore, that manuring does delay ripening, and in proportion to the amount of manure applied. Another characteristic of the crop at Amroha was the fact that during both the seasons the water content of the cane, after it had reached maturity, progressively decreased as long as the cane remained standing in the field.

It will thus be seen that the juice from canes at this station showed a low ash content and high purity and saline coefficients. Although the soil was not particularly good yet Amroha produced the best sugarcane found in the United Provinces and the Punjab. Co 313 in 1938-39 had a sucrose content of 12.3 and the others only slightly less. Although the cane produced was of very high quality yet the outturn per acre was low and thus suited the needs of the factories, but was not so satisfactory from the cultivator's point of view, where quantity matters most.

Bilari (1938-39 only)

The soil of this village in the Moradabad district is a medium loam, rich in nitrogen, organic matter and exchangeable calcium and has an acidic reaction. For average composition see below :—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1st foot	0.056	0.684	6.19	0.106	0.0740	0.100	15.8	22.0	62.20
2nd foot	0.0504	0.555	6.42	0.186	0.0468	0.060	30.0	20.0	50.0
3rd foot	0.0420	0.439	6.67	0.178	0.0521	0.060	35.2

Four varieties of cane were examined, namely Co 213, Co 312, Co 313 and Co 331 (Table VI). All were green manured with sunn-hemp with the addition of two maunds of ammonium sulphate and 15 maunds of castor-cake per acre with the result that the outturn of cane was heavy compared with other places in the United Provinces. This heavy manuring not only delayed maturity but also decreased the sucrose content and the purity coefficient and increased the ash compared with the unmanured Amroha canes. The recovery of sugar by the open-pan system as reported by the Sugar Experiment and Testing Station at Bilari was only 5.5.

Particulars	Percentage on cane							Glucose ratio	Purity coefficient	Saline coefficient	Yield per acre in maunds			Ripening period
	Juice	Sucrose	Glucose	Total sugars	Total solids	Non-sugars	Ash				Cane	Sucrose	Total solids	
Co 213	71.4	9.6	0.80	10.4	11.9	1.5	0.396	8.4	80.5	25.4	600.0	57.6	71.4	15/1 to 22/2
Co 312	70.0	11.0	0.63	11.6	12.7	1.1	0.255	6.4	86.5	43.0	850.0	93.5	108.1	20/2 to 22/2
Co 313	71.3	10.7	0.85	11.6	12.9	1.3	0.282	9.9	82.6	44.7	800.0	85.6	103.2	8/12 to 22/2
Co 331	68.3	9.9	0.59	10.5	11.4	0.9	0.270	4.1	86.4	37.6	1100.0	108.8	125.4	15/1 to 22/2
Average	70.2	10.3	0.71	11.0	12.2	1.2	0.281	7.2	83.9	47.7	837.5	86.3	100.2	..

TABLE VII

Analytical data for cane varieties, grown at Shahjahanpur (1938-39)

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 213	68.6	9.5	0.59	10.1	11.5	1.4	0.271	9.8	83.5	35.9	600.0	57.0	67.8	2/12 to 27/2
Co 312	66.1	10.4	0.51	10.9	12.0	1.1	0.247	5.9	87.0	42.1	800.0	83.2	96.0	2/12 to 27/2
Co 313	68.4	11.8	0.24	12.0	13.1	1.1	0.304	3.2	89.6	38.8	700.0	88.5	98.3	2/12 to 27/2
Co 331	66.4	10.1	0.45	10.6	11.6	1.0	0.238	6.4	86.2	47.6	800.0	80.8	92.8	2/12 to 27/2
Average	67.6	10.4	0.42	10.8	12.0	1.2	0.266	6.3	86.6	41.1	737.5	77.3	88.3	..

Shahjahanpur (1938-39 only)

The Shahjahanpur district is excellently suited for sugarcane having annual rainfall of about 40 in. and a good acid sandy loam soil, low in water soluble salt content. For average composition see table below :—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0448	0.400	6.24	0.072	0.0637	0.076	8.0	8.40	83.6
2nd foot	0.0364	0.362	6.40	0.132	0.0498	0.080	16.2	34.80	48.9
3rd foot	0.0280	0.324	6.67	0.132	0.0310	0.080	18.0	14.20	67.8

Four varieties of cane were examined, viz. Co 213, Co 312, Co 313 and Co 331 (Table VII) which were green manured with sunn-hemp plus two maunds of castor-cake and one maund of ammonium sulphate. The outturn of cane which was of good quality, was greater than that from Amroha and Salim but inferior in quality to the Amroha cane. The yield was from 600 to 800 maunds per acre and all varieties matured early in December.

The Punjab

The Karnal and Rohtak districts are regarded as the best sugarcane growing tracts in the Punjab, the climate being more suitable for cane than anywhere else in the province, with the result that in normal years high yields are obtained. Four localities were selected for the investigation, viz. Tharua, Jaukhli, Rohat (near Sonapat) and Karnal.

Tharu

It will be seen from the table below that the soil of this village is a loam, rich in organic matter, total nitrogen, exchangeable calcium and water soluble salts. The soil is acidic in reaction and becomes more so with increasing depth. It has the following average compositions :—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0840	1.035	6.75	0.176	0.081	0.200	32.2	23.3	44.5
2nd foot	0.0588	0.484	6.30	0.248	0.042	0.156	30.5	25.4	44.1
3rd foot	0.0532	0.469	6.30	0.232	0.042	0.140	32.8	22.7	44.5

Particulars	Percentage on cane							Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds			Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				Cane	Sucro- se	Total solids	
Co 244 : 1937-38	71.0	10.3	0.55	10.9	12.0	1.1	0.370	5.3	85.8	27.5	750.0	77.2	90.0	15/2
1938-39	65.8	8.9	0.75	9.7	11.0	1.3	0.341	8.9	81.0	25.1	800.0	71.2	88.0	..
Co 312 : 1937-38	73.2	6.1	1.30	7.4	8.4	1.0	0.687	21.1	72.2	10.1	850.0	51.8	71.4	16/1
1938-39	64.3	9.9	0.48	10.4	11.8	1.4	0.331	4.8	83.0	30.0	850.0	84.10	100.3	..
Co 313 : 1937-38	72.0	9.4	0.62	10.0	11.2	1.2	0.599	6.6	83.9	15.6	810.7	76.1	90.7	15/2
1938-39	64.9	10.1	0.41	10.5	12.0	1.5	0.452	3.8	83.8	23.8	800.0	80.0	96.0	..
Co 331 : 1938-39	63.0	8.6	0.79	9.4	10.9	1.5	0.439	9.0	78.9	20.2	875.0	75.2	93.4	..
Average : 1937-38	72.1	8.6	0.82	9.4	10.5	1.1	0.545	11.1	80.6	17.7	803.6	68.3	84.0	..
1938-39	64.4	9.4	0.61	10.0	11.4	1.4	0.391	6.6	81.7	24.7	831.0	77.8	87.97	..

As already noted the crop suffered particularly severely at this place pyrilla in 1937-38, hence the analytical data regarding the composition of cane cannot be regarded as normal. Of the three varieties, viz. Co 244, Co 313 (Table VIII) examined in 1937-38, Co 312 gave the highest yield, the lowest sucrose content, and had the highest ash content, hence a low saline coefficient.

In the following season, however, due to a higher sucrose and lower ash content the saline coefficient was the highest of all the varieties examined where in the Punjab during the two seasons, although the figure was of the same order as that obtained at Meerut which provided the poorest quality of cane in the United Provinces. The higher sucrose was due to the absence of pyrilla, but it is difficult to explain why a low ash content was obtained.

The average yield for the two seasons, however, was almost as good as that obtained at Karnal.

Rohat Harsana

The soil of this village as shown below is a sandy loam and the lightest of all the soils examined in the Punjab during the survey :—

Percentage on air-dried soil

Depth	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
1st foot	0.0728	0.776	6.94	0.158	0.028	0.180	18.1	20.4	61.5
2nd foot	0.0560	0.484	6.28	0.182	0.032	0.220	23.1	19.5	57.4
3rd foot	0.0525	0.371	6.14	0.218	0.002	0.240	25.1	17.6	57.3

The soil is rich in total nitrogen, organic matter, exchangeable calcium and has an acid reaction.

Five varieties of cane were examined, viz. Co 213, Co 285, Co 301, Co 312 and Co 313 in the first season, and four in the second, viz. Co 244, Co 312, Co 313 and Co 331 (Table IX). In 1937-38 as a result of pyrilla none of the cane matured. Nevertheless, the outturn was very high, Co 312 with 815.5 maunds per acre, giving the best yield. The quality of the cane, however, was naturally very poor.

In 1938-39, all the four varieties examined matured early in December the sucrose content of Co 331 (10.5 per cent) being the highest while that of Co 313 the lowest (9.7). The quality of cane resembled that grown at Meerut with a high ash content and a low saline coefficient. Yields were approximately 750 maunds per acre.

Particulars	Percentage on cane						Glucose ratio	Purity coefficient	Saline coefficient	Yield per acre in maunds			Ripening period
	Juice	Sucrose	Glucose	Total sugars	Total solids	Non-sugars	Ash			Cane	Sucrose	Total solids	
Co 213 : 1937-38	71.9	7.7	0.62	8.3	9.9	1.6	0.646	8.1	79.3	11.9	570.0	40.5	50.4 11/2
Co 285 : 1937-38	68.3	7.1	0.50	7.6	9.4	1.8	0.707	7.0	78.9	10.0	700.0	60.8	73.5 11/2
Co 301 : 1937-38	73.5	8.5	0.77	9.3	10.8	1.5	0.680	9.1	72.0	12.5	775.0	65.8	83.7 14/2
Co 312 : 1937-38	72.8	8.8	0.56	9.4	10.8	1.4	0.465	6.4	81.5	18.4	815.5	71.7	88.1 2/2 to 24/2
1938-39	63.9	10.1	0.39	10.4	11.7	1.3	0.476	4.0	84.8	21.2	820.0	82.8	95.9 1/12 to 12/2
Co 313 : 1937-38	69.6	9.1	0.47	9.6	10.9	1.3	0.628	5.2	83.5	16.6	790.4	71.9	86.1 2/12 to 24/2
1938-39	60.1	9.7	0.30	10.0	11.6	1.6	0.457	3.1	84.0	20.7	760.0	73.7	88.2 1/2 to 12/2
Co 331 : 1938-39	63.1	10.5	0.26	10.8	12.2	1.4	0.415	2.5	85.8	23.1	690.0	72.4	84.2 1/12 to 12/2
Co 244 : 1938-39	62.6	10.4	0.279	10.7	12.1	1.4	0.470	2.4	85.9	22.1	780.0	81.1	94.4 1/12 to 12/2
Average : 1937-38	71.2	8.2	0.58	8.8	10.2	1.4	0.623	7.2	79.7	13.9	746.2	62.8	73.5
1938-39	62.4	10.2	0.31	10.5	11.9	1.4	0.439	3.0	85.1	21.8	762.5	77.9	90.7

TABLE X

Analytical data for cane varieties grown at Villupuram

Particulars	Percentage on cane					Glu- cose ratio	Purity coeffi- cient	Saline coeffi- cient	Yield per acre in mounds		Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids				Cane	Sucro- se	
Co 244:	79.1	9.9	0.55	9.7	13.9	8.4	83.5	13.5	555.0	50.5	20.1 to 25.2
	66.1	19.3	0.21	10.5	12.0	2.3	83.1	21.4	300.0	61.8	7.12 to 19.2
Co 312:	73.3	8.7	0.65	9.4	10.4	7.5	82.5	20.4	628.3	54.7	17.2 to 25.2
	67.6	10.9	0.16	11.2	12.5	1.8	87.6	19.4	700.0	70.3	7.12 to 19.2
Co 313:	70.5	8.8	0.47	9.3	10.9	5.3	81.1	13.9	615.7	54.2	25.2
	64.5	10.0	0.20	10.2	11.7	1.9	85.0	17.3	650.0	65.0	7.12 to 19.2
Average: 1937-38	71.4	8.9	0.56	9.5	10.8	6.3	82.4	15.9	599.7	53.1	64.5
1938-39	66.1	10.4	0.28	11.0	12.0	2.0	86.2	19.4	650.0	67.7	75.0

is a typical village of the *khadar* tract, whose soil is a medium loam characterized by a highly alkaline reaction, low exchangeable calcium and water-soluble contents. The average composition is shown below :—

Percentage on air-dried soil

	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
	0.0728	0.986	7.97	0.072	0.037	0.220	16.3	31.6	52.1
	0.0588	0.807	8.11	0.074	0.014	0.220	20.1	37.2	42.8
	0.0476	0.524	7.91	0.060	0.019	0.240	22.6	42.7	34.7

is soil had the lowest exchangeable calcium content of any of the soils in the Punjab—a fact which appears to be reflected in the ash content of the cane juice particularly in the case of Co 244 which was 0.71 per cent at Jaukhli but only 0.37 per cent at Tharu. Compared with other soils in the Punjab even in the abnormal season of 1937-38, Jaukhli produced only medium quality canes (Table X). In the following season all the varieties matured early and had a moderately good sucrose content, high ash content and a low saline coefficient, thus resembling the other canes in the area.

Canes examined at Karnal were taken from the Government Agricultural Station which, as will be seen from the data below, has a clay loam soil rich in organic matter, total nitrogen and exchangeable calcium. The concentration of water-soluble salts, however, is rather high and the soil reaction acidic.

Percentage on air-dried soil

	Total nitrogen	Organic matter	pH	Exchangeable calcium	Available P_2O_5	Water-soluble salts	Clay	Silt	Sand
	0.0756	1.000	6.50	0.190	0.014	0.160	23.0	31.5	45.5
	0.0483	0.936	6.42	0.220	0.037	0.110	29.1	29.2	41.8
	0.0462	0.855	6.22	0.228	0.019	0.130	31.0	28.5	40.5

Of the four varieties examined in 1937-38, viz. Co 285, Co 312, Co 313 and Co 331 (Table XI), Co 312 and Co 331 were badly attacked by pyrilla, yield only 5 per cent and 4.5 per cent sucrose on cane, respectively. Co 313 was the highest yield. All the four varieties had low purity coefficients and low glucose ratios, both the sucrose content and saline coefficients being low. The crop showed a tendency to lodge. In the following season all the four varieties examined, viz. Co 213, Co 312, Co 313 and Co 331 matured in the middle of December. The ash content of the juice was very high, but as regards nutrition, the cane was in no way inferior, to that on the Government Farm Meerut, where improved methods of cultivation were followed. The sucrose content and ash were equal at both these localities, but the weight of cane produced per acre was higher at Karnal by 100 maunds than at Meerut.

GENERAL OBSERVATIONS

Ripening of sugarcane

It will be seen from Tables II-XI that the yield of stripped cane in the Punjab was about 40-50 per cent higher than in the United Provinces, which shows that the Punjab canes attained a more vigorous and greater vegetative growth than those in the neighbouring province. Hence, they must remain in the field for a longer period before they are fully mature, since all factors which increase vegetative growth cause delay in ripening. Again we see that the Punjab soils are richer in organic matter, total nitrogen and water-soluble salts and are much more heavily manured than the United Provinces. The number of irrigations given is also greater, but, taking into consideration the higher rainfall in the United Provinces, the total amount of water received by the sugarcane crop, both as irrigation and rain water, is usually the same. It is thus clear that, while most of the canes in the United Provinces will be fit for crushing early in the season, the same varieties will be unripe in the Punjab. If, therefore, the same varieties of cane are crushed in the two tracts at the same time, the United Provinces canes being fully ripe will naturally give a greater recovery.

Quality and yield of sugarcane

The data for the season 1937-38, relating to the quality and quantity of cane varieties which are common to the two tracts, are given in Table X.

It will be seen from these figures, that apart from Co 331 which gave a high total solids and sucrose per acre in the Punjab than in the United Provinces, all the varieties examined gave a greater outturn of stripped cane, sucrose and total solids in the Punjab. The quality of cane, however, as judged by the amount of sucrose and ash expressed as percentage on cane, purity coefficient and saline coefficient, was much poorer in the Punjab.

Similar data for the following season 1938-39 are given in Table XI. It will be observed that this was a better season for cane both in the United Provinces and the Punjab, specially in the latter where the cane crop benefited from better climatic condition and was also free from pyrilla, with the result that the sucrose percentage on cane was almost as good as in the United Provinces. Similarly the 'cane ratio' and net rendiment showed marked improvement. The yield of stripped cane per acre increased by over

Particulars	Percentage on cane						Purity coeffi- cient	Saline coeffi- cient	Yield per acre in maunds		Ripening period
	Juice	Sucro- se	Glu- cose	Total sugars	Total solids	Non- sugars	Ash				
Co 285 : 1937-38	75.5	7.7	1.44	9.1	10.1	1.0	0.505	76.9	15.5	613.3 47.2	61.9 27/12
Co 213 : 1938-39	65.0	10.0	0.36	10.4	12.0	1.6	0.579	83.1	17.3	650.0 65.0	78.0 9/12 to 23/2
Co 312 : 1937-38 1938-39	68.5 69.2	5.0 10.4	1.50 0.29	6.5 10.7	7.7 12.1	1.2 1.4	0.358 0.398	65.8 85.5	13.8 26.1	757.3 37.8 1265.0 131.6	58.3 155.1 28/2 9/12 to 23/2
Co 313 : 1937-38 1938-39	67.2 67.2	7.9 10.9	0.76 0.29	8.7 11.2	9.7 12.9	1.0 1.7	0.420 0.495	81.9 84.0	18.8 22.0	612.1 48.3 820.0 89.4	59.3 105.8 28/2 19/12 to 23/2
Co 331 : 1937-38 1938-39	72.3 66.6	4.5 10.0	2.18 0.48	6.7 10.5	6.9 11.9	1.2 1.4	0.509 0.462	65.3 83.5	8.4 21.8	551.3 24.8 1000.0 100.0	38.0 119.0 28/2 9/12 to 23/2
Average: 1937-38 1938-39	70.9 66.9	6.3 10.3	1.47 0.34	7.8 10.6	8.6 12.2	1.1 1.6	0.448 0.481	72.5 84.1	14.1 21.8	633.5 39.5 934.0 96.5	54.4 114.0

TABLE XII
Average composition of sugarcane in the Punjab and the United Provinces and other relative data, 1937-38

Province	Percentage on sugarcane						Mauds per acre				
	Sucrose	Glucose	Total sugars	Total solids	Non-sugars	Ash	Purity coefficient	Saline coefficient	Stripped cane	Sucrose	Total solids
United Provinces	11.0	0.53	11.5	12.6	1.1	0.42	87.0	27.9	303.0	33.0	37.6
Punjab	7.7	0.49	8.2	9.7	1.5	0.65	79.3	11.9	570.0	43.9	56.4
United Provinces	10.7	0.57	11.3	12.2	0.9	0.33	87.4	32.4	437.1	46.1	53.0
Punjab	9.7	0.55	10.3	11.5	1.2	0.47	84.6	20.5	652.5	63.3	75.2
United Provinces	9.7	0.75	10.5	11.5	1.0	0.26	84.1	37.5	559.2	53.0	60.1
Punjab	8.0	1.0	9.0	10.3	1.3	0.52	75.5	15.4	762.7	54.0	71.0
United Provinces	10.9	0.34	11.2	12.3	1.1	0.33	83.5	28.7	474.6	67.9	57.9
Punjab	8.5	0.58	9.1	10.3	1.2	0.52	82.6	16.2	682.2	83.5	75.8
United Provinces	9.4	0.65	10.1	11.2	1.1	0.33	83.6	28.7	533.0	49.4	56.7
Punjab	4.5	2.18	6.7	8.0	1.3	0.51	65.2	8.4	741.0	44.0	54.0

Average relative data

Particulars	United Provinces	Punjab
per cent on cane	10.3	7.7*
per cent on cane	0.342	0.539
coefficient	86.1	77.5
coefficient	30.1	14.3
ratio	11.7	19.2
adiment or available sugar	8.55	5.2
	Maunds	Maunds
aged cane per acre	461.3	643.9
per acre	49.9	53.9
solids per acre	53.4	63.3

A low figure due to pyrrilla

unds in the Punjab and by 150 maunds in the United Provinces, this increase being due to the fact that the figures for Bilari and Shahjahan- where large quantity of manure are generally applied, were included in the average for this season.

On considering the relative features which have been described in regard to growth of cane obtained in the two provinces, both from the quantitative and qualitative aspects, the most important point perhaps to bear in mind is that the ash content and 'solids non-sugars' in the juice from the Punjab are considerably higher than in those from the United Provinces. This is doubt, due to the higher concentration of water-soluble salts in the Punjab soils, whereas in the United Provinces the soils are lighter and thus more permeable. The cane ratio or the number of tons of cane required to produce a ton of sugar is higher in the Punjab than in the United Provinces. The ratio is a function not only of the concentration of sucrose in the juice but also of its purity, the latter being the ratio of sucrose to total solids in the cane. The greater the proportion of mineral matter, the more difficult it is to recover sugar in the process of manufacture. Nitrogenous manuring tends to increase the percentage of impurities in the juice and consequently the cane yield to a greater degree than might be expected solely from a consideration of differences in sucrose content. Consequently a relatively large percentage of sugar remains unrecovered in the factory when cane is grown under heavy manuring. However, some of these factors counterbalanced each other when comparing the sugar recovery from the canes of the western United Provinces with the south-eastern Punjab, and it was found on balance that the difference was not greater than about one per cent.

In order to gain more accurate data as to how far different nitrogenous manures affect the composition of cane juice, Co 312 was manured at the Shahjahanpur Sugarcane Research Station with 100 lb. and 200 lb. of nitrogen in the form of castor-cake, ammonium sulphate and farmyard manure under two, four and six irrigations. The data from these investigations are given in Tables XIV-XVI, from which it will be seen that the sucrose content

TABLE XIII

Average composition of sugarcane in the Punjab and United Provinces and other relative data, 1938-39

Province	Percentage on sugarcane						Purity coefficient	Saline coefficient	Maunds, per acre		
	Sucrose	Glucose	Total sugars	Total solids	Non-sugars	Ash			Stripped cane	Sucrose	Total solids
United Provinces	10.2	0.55	10.8	12.1	Co 213 1.3	0.332	84.3	397.0	40.2	48.5	
Punjab	10.0	0.37	10.4	12.0	1.6	0.567	83.3	610.0	61.0	73.0	
United Provinces	10.9	0.22	11.1	12.3	Co 244 1.2	0.286	88.6	400.0	43.6	49.2	
Punjab	10.7	0.23	10.6	12.1	1.5	0.479	85.9	723.0	75.2	87.5	
United Provinces	10.6	0.50	11.1	12.2	Co 312 1.1	0.267	86.9	722.0	76.3	87.8	
Punjab	10.5	0.29	10.8	12.1	1.3	0.488	86.8	909.0	95.4	110.0	
United Provinces	11.3	0.40	11.7	13.0	Co 313 1.2	0.331	86.9	578.0	65.3	75.1	
Punjab	10.3	0.27	10.6	12.1	1.6	0.511	85.1	757.0	78.0	91.6	
United Provinces	10.4	0.44	10.8	12.0	Co 331 1.1	0.290	86.7	764.0	79.4	91.7	

Average relative data

Particulars	United Provinces	Punjab
per cent on cane	10·7	10·4
per cent on cane	0·314	0·504
coefficient	87·0	85·9
coefficient	34·1	20·6
ratio	11·4	12·0
ndiment or available sugar	9·18	8·35
	Maunds	Maunds
ed cane per acre	611·1*	776·2
per acre	63·9	78·8
solids per acre	73·6	90·3

The average outturn was higher this season as Bilari and Shahjahanpur were included and large quantities of manure are applied.

depressed and ripening delayed as the quantities of nitrogen applied increased. No particular increase in the ash content of cane manured with ammonium sulphate was found, but the increase was significant when farmyard manure was employed. Generally speaking it may be stated with reasonable expectation that the amount of solids non-sugars in cane may be expected to increase in proportion to the amount of manure employed.

GENERAL CONCLUSIONS AS TO THE CAUSES RESPONSIBLE FOR THE ALLEGED INFERIOR QUALITY OF PUNJAB CANES COMPARED WITH UNITED PROVINCES CANES FOR SUGAR PRODUCTION

The general results of the survey indicate that the poor quality of the Punjab canes is mainly due to the composition of the soil of the province—conclusion which has stimulated investigators to see to what extent the ash content of the juice can be lowered by altering the composition of the soil by the application of appropriate chemicals such as gypsum. There is tentative evidence already that gypsum may be efficacious for this purpose in such soils.

In regard to operations in sugar factories it will be seen from the appendix that the mineral content of the clarified juice is lowered to a greater extent if the carbonation process rather than the sulphitation process is employed, and as the Punjab has plenty of lime available this industry should flourish in certain localities provided judicious agricultural operations are followed, due consideration being taken of the likelihood of frost in the localities selected and the carbonation process followed in factories.

TABLE XIV
Sugarcane analysis

(Sugarcane Experiment Station, Shahjahanpur, 1939)

Date of analysis	Variety of cane	Description of sample	Percentage on juice					Glucose ratio	Purity coefficient	Saline coefficient	Percentage on cane			Remarks
			Sucrose	Glucose	Total solids	Non-sugars	Mineral matter				Juice	Sucrose	Total solids	
22/1	Co 312	Control N ₁	13.8	1.04	16.7	1.9	0.412	7.3	82.9	33.4	66.7	9.2	11.1	Six irrigations
"	"	Castor-cake N ₂	13.3	1.17	16.4	1.9	0.345	8.8	81.6	40.4	66.7	8.9	10.9	100 lb. nitrogen
"	"	" N ₂	12.6	1.65	16.1	1.9	0.345	12.9	78.0	38.6	67.7	8.5	10.9	200 lb. nitrogen
"	"	Control N ₁	14.8	0.64	17.3	1.9	0.430	3.9	86.1	38.5	67.7	9.9	11.5	
"	"	Ammonium sulphate N ₂	13.5	1.59	16.5	1.6	0.300	10.2	80.9	55.1	68.6	9.3	11.5	100 lb. nitrogen
"	"	"	12.2	1.75	15.3	1.8	0.214	14.3	79.8	56.2	68.1	8.9	11.3	200 lb. nitrogen
"	"	Control	14.3	0.77	16.9	1.8	0.397	5.6	84.5	46.2	68.8	9.8	11.6	
"	"	F. Y. M. N ₂	13.0	0.90	16.6	1.8	0.360	6.2	83.8	43.1	66.6	9.3	11.1	100 lb. nitrogen

(Sugarcane Experiment Station, Shahjahanpur, 1939)

Date of analysis	Variety of cane	Description of sample	Percentage of juice					Glucose ratio	Purity coefficient	Saline coefficient	Percentage of cane			Remarks
			Percentage of juice				Juice				Sucrose	Total solids		
			Sucrose	Glucose	Total solids	Non-sugars							Mineral matter	
23/1	Co 312	Control N ₁	14.7	1.03	13.1	2.4	0.325	7.0	81.4	45.5	65.0	9.6	11.8	Four irrigations
"	"	Castor-cake N ₂	12.7	1.63	16.0	1.7	0.443	12.9	79.0	28.5	65.6	8.3	10.5	100 lb. nitrogen
"	"	" N ₂	13.5	1.20	16.8	2.1	0.389	8.9	80.5	34.8	67.3	9.1	11.3	200 lb. nitrogen
"	"	Control N ₃	14.2	0.80	17.4	2.4	0.429	5.7	81.4	33.0	65.0	9.2	11.3	"
"	"	Ammonium sulphate N ₂	12.7	1.58	16.1	1.8	0.399	12.4	79.4	35.6	66.6	8.5	10.7	100 lb. nitrogen
"	"	Ammonium sulphate N ₂	13.0	1.35	16.4	2.0	0.292	10.2	78.8	46.3	63.8	8.9	11.3	200 lb. nitrogen
"	"	Control N ₁	14.3	0.63	16.9	2.0	0.456	4.3	85.1	31.4	67.7	9.7	11.4	"
"	"	F. Y. M. N ₂	13.6	1.0	17.1	2.5	0.451	7.2	79.1	30.0	64.3	8.7	11.0	100 lb. nitrogen
"	"	F. Y. M. N ₂	13.8	0.66	17.1	2.6	0.501	5.0	80.8	27.6	70.0	9.7	12.0	200 lb. nitrogen

Another point of considerable importance brought out during this survey is the fact that, although manuring results in an increased yield of cane, it tends to lower the quality of the juice, and it appears, therefore, that measures aimed at increasing the yield of cane should be carefully controlled in conjunction with the quality of cane obtained so as to ensure that the latter is not seriously affected.

The quality and yield of the cane crop depend mainly on : (i) climate, (ii) nature of soil and (iii) agricultural operations. It is a well-known fact that in order to produce one pound of dry matter in an arid region more water is required than to produce the same amount of crop in a humid climate. I assume, therefore, the concentration of the soil solution (in more general terms the amount of water-soluble salts present in the soil) to be the same in the two cases, it is likely that the crop grown in a dry climate will take up and retain a greater amount of plant food material owing to increased transpiration.

Again, the concentration and nature of the soil solution will depend respectively upon the amount and nature of soluble salts present in any particular soil. Furthermore, the application of manures as followed in the Punjab without the corresponding increase in the amount of water applied appears to be responsible for an increase in the amount of mineral matter in the cane crop. Considering all the factors, it appears that the greater amount of ash present in the juice from the Punjab canes is the main cause of their alleged inferiority. This is confirmed by the following figures (1937-38) relating to Sonapat in the Punjab where the cane crop was raised under ordinary zemindari conditions, Bishokhar and Meerut in the United Provinces, where improved methods of cultivation depending upon liberal irrigation and application of sufficient manures were followed.

It will be seen from the figures of purity and saline coefficients for different varieties of cane grown at various stations in the two provinces (Tables XII and XIII) that the canes from the western United Provinces are generally superior to those obtained from the south-eastern Punjab. Nevertheless, as shown in Table XIII, those from Bishokhar and Meerut were not as good as those from Sonapat, since the latter were grown under improved methods of cultivation.

It seems that attempts to increase the yield of cane beyond a certain limit are likely to result in deterioration of quality unless suitable varieties of cane are evolved which will maintain their quality as quantitative production

Percentage on cane

Variety	Locality	Juice (per cent)	Sucrose	Glucose	Total solids	Ash	Glucose ratio	Purity	Saline coefficient
3	Sonapat . . .	62.2	12.0	0.1	13.4	0.390	1.0	89.8	30.8
	Bishokhar . . .	69.0	11.5	0.15	13.2	0.528	1.3	86.9	21.7
	Meerut . . .	69.0	10.6	0.23	11.8	0.501	2.1	90.1	21.2
0 1	Sonapat . . .	65.6	10.6	0.30	12.1	0.360	3.0	87.6	29.5
	Bishokhar . . .	71.1	9.6	0.55	11.6	0.404	5.6	82.8	23.8
	Meerut . . .	69.3	9.4	0.36	11.2	0.479	3.8	83.8	19.5

is increased. In this connection mention may be made of an interesting observation made at Amroha, which produced the best canes of any in the Provinces or the Punjab. It was found that the amount of mineral which the cane crop is able to absorb during its growth reaches a maximum at maturity, and if the crop remains longer in the field the mineral matter remains stationary or decreases. In other words, once the crop is mature there is little likelihood of any further increase in the mineral content of the juice. Obviously, in this respect early-maturing varieties will have an advantage over those which mature late, although their yields must not be too low. Attempts should be made to evolve varieties which will give high yields and yet mature early. Further, such varieties should maintain quality over the entire crushing period as long as they remain standing in the field.

SUMMARY

It was found during the sugarcane seasons 1935-36 and 1936-37 that the recovery of white sugar at the Sonapat Sugar Factory in the south-east Punjab from sugarcane grown in the surrounding areas was only 7.7 per cent, whereas cane imported from the neighbouring tracts across the river Jhelum in the United Provinces gave a recovery of 10.18 per cent. The present investigation was conducted with a view to ascertain the causes responsible for this wide difference.

A number of localities were selected in each of the two tracts and the varieties in each were analysed during the sugarcane seasons 1937-38 and 1938-39. The soil from the fields growing these canes was sampled at a number of places to a depth of 3 ft. and analysed for different constituents.

It was found that the composition of the soil in the two tracts differs considerably in the matter of organic matter, nitrogen and water-soluble salts, the Punjab soils being richer in these constituents. The United Provinces soils are inclined to be slightly more acidic and lighter than the Punjab soils.

The analysis of different varieties of cane shows that canes grown in the Punjab have a slightly lower sucrose and glucose content but contain more mineral matter in the juice than corresponding canes in the United Provinces. These differences are reflected in the higher purity coefficient and significantly higher saline coefficient of the cane from the United Provinces.

It has been found that the higher mineral matter in the juice of the Punjab canes is the main reason for the low recovery of white sugar in the Punjab.

It has been shown that the mineral matter in the juice of sugarcane does not increase after the canes have reached maturity. On the other hand, in certain cases the mineral content has been found to decrease after maturity. This indicates that early ripening varieties are better suited for the soil and climatic conditions of the Punjab. Efforts should, therefore, be directed to evolve such varieties and at the same time aim for higher yields.

The carbonation process is better suited than the sulphitation process for the manufacture of white sugar in the Punjab as the former reduces the mineral content of the mixed juice during clarification to an extent which is about four times as great as that obtained with the other.

ACKNOWLEDGEMENTS

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REFERENCE

Mehta, P. E. and Ramji Narain. (1936). Mineral matter in the juice of sugarcane and its effect on the recovery of white sugar, I. *Indian J. agric. Sci.* **6**, 1218

APPENDIX A

In the manufacture of white sugar two of the most important methods employed for purification of sugarcane juice in the factory are :—(i) the sulphitation process, (ii) the carbonation process. In the first process lime and sulphur are used as clarifying agents and in the second carbon dioxide and lime. The latter process is more expensive than the former on account of the greater quantity of lime used but the increase in cost is compensated for by a higher recovery of sugar of better quality. Since the juice of sugarcane in the Punjab is characterized by the presence of mineral matter in relatively high amounts, the process of clarification which will yield clarified juice with a lower mineral content will naturally be better suited to conditions in the Punjab. Data were collected during the survey to ascertain which of the two processes is more suited for the Punjab. Two factories working within a few furlongs of each other were selected for carrying out these data and the figures obtained are given below :—

Sulphitation process

Particulars	Brix	Pol	Purity	
Mixed juice	15.5	11.7	75.7	} Average of the 1st fortnight
Clarified juice	17.1	13.2	77.0	
Mixed juice	16.0	12.2	76.5	} Average of the 2nd fortnight
Clarified juice	18.2	14.2	78.1	
Mixed juice	16.3	12.6	77.3	} Average of the 3rd fortnight
Clarified juice	18.8	14.8	78.6	
Mixed juice	16.6	13.1	78.9	} Average of the 4th fortnight
Clarified juice	18.1	14.4	79.7	

Average increase of purity from mixed juice to clarified juice is equal to 1.25

Carbonation process

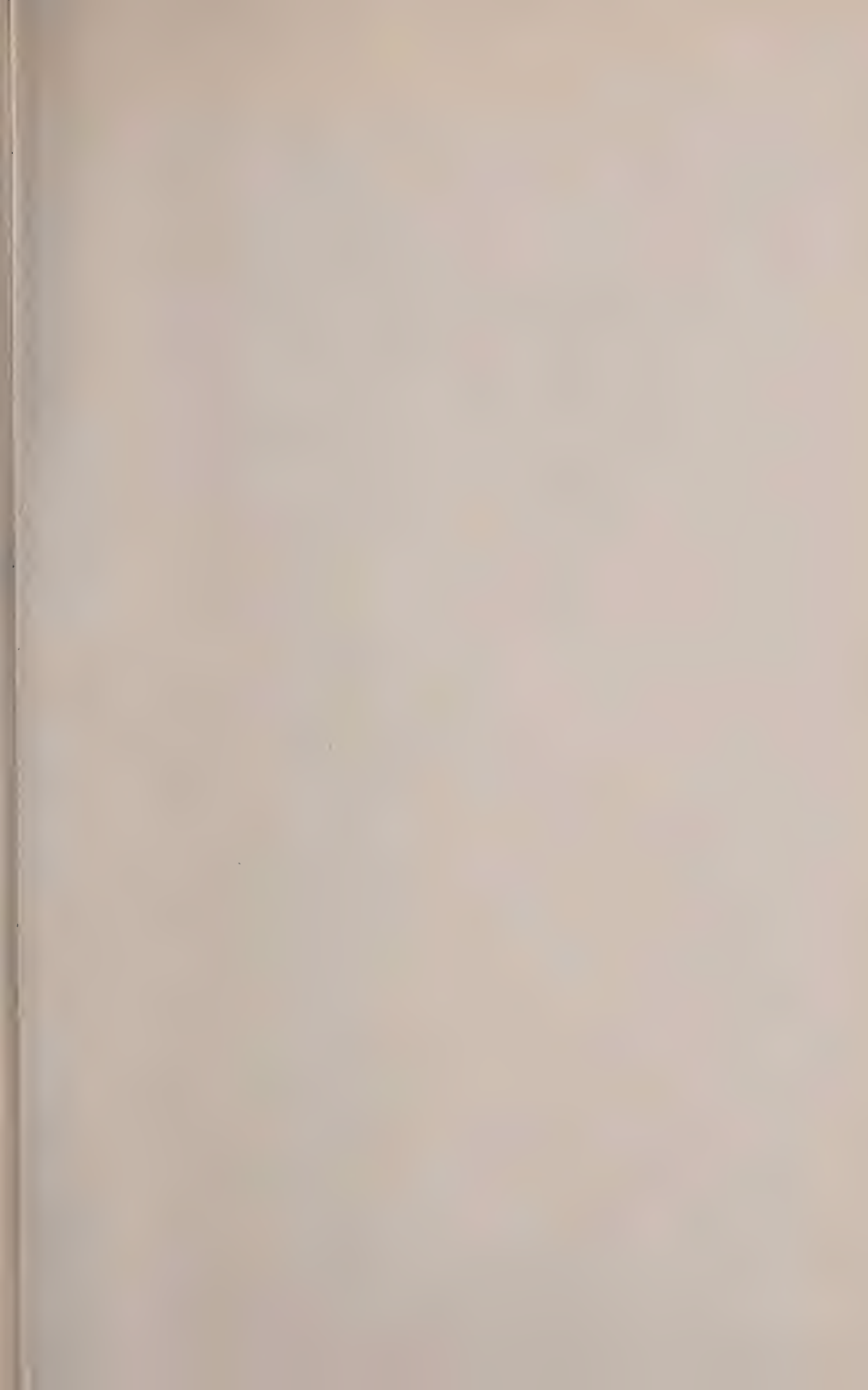
Particulars	Brix	Pol	Purity	
Mixed juice	15.0	11.3	75.2	} Average of fortnight
Clarified juice	13.4	10.5	78.0	
Mixed juice	15.9	12.1	76.3	} Average of fortnight
Clarified juice	14.3	11.3	78.2	
Mixed juice	16.4	12.6	77.1	} Average of fortnight
Clarified juice	15.3	12.2	79.3	
Mixed juice	16.5	12.9	78.3	} Average of fortnight
Clarified juice	15.5	12.6	80.9	

Average increase of purity 2.4

These figures indicate that there is a greater elimination of non-saccharine from the juice by the carbonation process than with the other. The following further show that the elimination of ash is also much greater by the former process being almost four times as great as in the latter.

Particulars	Ash per cent juice
<i>Sulphitation process</i>	
Mixed juice	0.530
Clarified juice	0.474
Mixed juice	0.565
Clarified juice	0.487
<i>Carbonation process</i>	
Mixed juice	0.540
Clarified juice	0.317
Mixed juice	0.548
Clarified juice	0.275

These data show that the carbonation process is better suited for Punjab than the sulphitation process





STUDIES ON SOME FUNGI ISOLATED FROM 'BLACK POINT' AFFECTED WHEAT KERNELS IN THE CENTRAL PROVINCES

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(With Plate XXIX and eight text-figures)

In a previous publication [Dastur, 1933] it was mentioned that from 'black point' infected wheat kernels, though they all look alike, more than one fungus has been isolated when incubated under aseptic conditions. The present paper deals with a study of some of those fungi which have not been as yet recorded on wheat kernels.

The 'black point' infected seed was surface-sterilized by a brief soak in a 0.1 per cent solution of corrosive sublimate or in rectified spirit and was planted on moist sterilized filter paper in a sterilized petri dish.

In a majority of cases not more than one fungus at a time was found associated with the diseased seed. In those few cases which gave a mixed flora the mixture included one or more of the following :—*Aspergillus* sp., *Mucor* sp., *Fusarium* sp., *Chaetomium* sp., *Alternaria* sp., *Botrytis* sp.

Botrytis tritici n. sp. Dast.

In some rare cases an ascigerous fungus was isolated from 'black point' affected wheat kernels; 'black point' affected seeds are usually viable; but those in which the blackening of the embryo end was caused by this ascigerous fungus failed to germinate; as the number of isolations of this fungus has been small it is not possible to conclude that the kernel diseased by this fungus is not viable. A few days after the surface-sterilized seed was planted on moist filter paper in a petri dish under sterile conditions minute wartlike protuberances appeared on the seed. Later the pericarp ruptured and globose or subglobose black bodies, the perithecia, became distinctly visible; similar bodies were also seen scattered amongst the hyphae which had spread from the diseased seed to the filter paper. No other organism was found associated with this fungus. On the filter paper the perithecia are scattered; on the seed they are at first scattered but later they become crowded together forming a sticky, mucous, brittle, crust-like mass. The perithecia on the kernels are not developed in its tissues but are formed on the outside of the pericarp partially covered by a cob-web of dark coloured hyphae. There is no development of a stalk (Plate XXIX, fig. 1). On agar media and sterilized wheat stalks, bran or bran the perithecia are similarly developed; they are erumpent, and solitary or gregarious. They have generally a prominent neck especially when formed on the 'black point' affected kernel (Plate XXIX, fig. 2), but at the base the neck is absent. This is particularly so in the case of perithecia

developed on culture media (Plate XXIX, fig. 3). They are globose, sub- or flask shaped, usually glabrous; but at times there is a development of undifferentiated hyphae; the perithecia are black in colour, neck when present is cylindrical and has a fringe of hyaline cells at the tip. The walls of the perithecia are thick but fragile; they break even under slight pressure. The perithecia with the beak measure $219.8-596.6 \times 172.1-406.1 \mu$; the body measures $204-550 \mu$; the beak measures $53.1-235.9 \times 53.1-123.9 \mu$.

The asci are numerous and embedded in filiform paraphyses (Plate XXIX, fig. 4). In a mass, asci and paraphyses have a greenish tinge but individually they are hyaline. The asci are usually long and narrow (Plate XXIX, fig. 5), but at times they are short and broad; they are straight or slightly curved; they are more or less clavate in shape; the long asci are usually pedicellate (Plate XXIX, fig. 6); whereas the short ones are sessile with a rounded base (Plate XXIX, fig. 7); they measure $79.8-228 \times 13.3-34.2 \mu$; usually they measure $121.6-203.2 \times 15.2-22.8 \mu$. They contain eight ascospores.

Paraphyses are generally inconspicuous, though numerous, and surround the asci; they are very slender, up to 1.7μ , in width; they are frequently branched, occasionally dichotomously; they are multiseptate; not constricted at the septum; the apex is rounded and slightly broader than the rest of the body of the paraphysis.

The ascospores are coiled in a close helix (Plate XXIX, fig. 8); they usually escape from the asci through an opening formed at the apex by the dissolution of the apical part (Plate XXIX, fig. 5). At times they escape from the end of the ascus, and occasionally from both the ends simultaneously. When the helix of the ascospores emerges through the opening it gets uncoiled; when the whole mass of coiled ascospores is out of the ascus they are usually completely free from each other and are scattered some distance away from the asci. At times only a few ascospores escape from the helix and the remaining ascospores are still confined in a loose helix. The ascospore is thin, long and arcoid or horse-shoe or wavy or contorted in shape (Plate XXIX, fig. 9); it is straight; and, therefore, its length cannot very accurately be measured; the apex is slightly rounded; the basal part tapers gradually and the end is pointed; it is hyaline in colour, but rarely has an olivaceous tinge; it is many-septate, 4-12; at times there is a constriction at the septum; the ascospores measure $125.4-301.6 \times 3.8-7.6 \mu$; they germinate readily; germ-tubes are developed from any or all segments.

In one of the progenies of a culture started from a single ascus, conidia resembling those of *Helminthosporium* were developed. These conidia are light brown or honey coloured, and 5 to 9 septate. In shape they are generally elliptical and straight or slightly curved; they are not variable in size. They are rounded at both ends, have a basal scar and are never forked. They measure $45.6-83.6 \times 11.4-15.2 \mu$. The germination is bi-polar; the germ-tube is firm.

Single spore cultures of this *Helminthosporium* gave only the conical stage on Glucose agar and Rice meal agar; on two per cent plain agar no conidia were not developed; but only empty globular or flask shaped bodies, black or brown in colour, resembling the perithecia described above,

ed ; on sterilized wheat grains and wheat bran perithecia and conidia were developed.

Conidia from a single spore culture of this *Helminthosporium* were used for inoculating ears of wheat plants grown in pots. The inoculum was placed in the glumes after the flowers had set. The glumes of the grains of the inoculated plants showed typical symptoms of infection. The glumes had the characteristic tobacco coloured or blackish brown coloured lesions and the kernels showed the typical 'black point'. When these glumes and kernels were tested under aseptic conditions on culture media the ascigerous fungus was isolated. The asci measured $126.0-231.0 \times 15.75-21.0 \mu$; the ascospores measured $157.5-345.4 \mu$.

Surface-sterilized wheat and rice grains were inoculated with this single-spore culture of *Helminthosporium*, and incubated in sterilized moist chambers. The perithecial stage was not developed but the hyphae produced *Helminthosporium* conidia in large numbers. The conidia on these wheat grains measured $83.6 \times 11.4-15.2 \mu$; the number of septa varied from five to eleven. Conidia on rice grains were similar to those found on cultures of the ascigerous fungus and measured $38.0-58.8 \times 7.6-15.2 \mu$. The septa varied from five to nine.

TAXONOMY

The genus *Ophiobolus* Riess, in the broad Saccardian sense, can be readily divided into two distinct series, the helicoid and the non-helicoid ascigerous series according to the arrangement of the ascospores in the asci. The known perfect stages of the graminicolous *Helminthosporia* belong to the helicoid series. Drechsler [1934] has shown that these graminicolous *Helminthosporia* have characteristics which are sufficiently distinctive and constant to be grouped together for purposes of classification. He, therefore, has removed this helicoid series from the original genus *Ophiobolus* and has placed it in a new genus, *Cochliobolus*, a name which indicates the helicoid arrangement of the ascospores. The type species is *C. heterostrophus* (Syn. *Ophiobolus heterostrophus* Drechs.), the ascigerous stage of *Helminthosporium maydis* Nishikado et al. on *Zea mays*. Therefore, according to Drechsler, the following members of the helicoid ascigerous series, *Ophiobolus miyabeanus* Ito et al. (Syn. *H. bayashi* (Syn. *H. Oryzae*. Breda de Hann) on *Oryza sativa*; *O. sativus* (Syn. *H. K. et B.*) Ito et Kuribayashi (Syn. *H. sativum* Pammel, *H. acrothecioides* Drechs.) on *Hordeum sativum* Jess. and *Triticum vulgare*; *O. setariae* Sawada et Kuribayashi (Syn. *H. setariae* Sawada) on *Setaria italica*, *S. glauca* and *S. viridis*; *O. kusanoi* Nishikado (Syn. *H. kusanoi* Nishikado) on *Stenotaphrum secundatum* major would now be renamed *Cochliobolus miyabeanus* (Ito et Kuribayashi) Drechs., *C. sativus* (Ito et Kuribayashi) Drechs., *C. setariae* (Ito et Kuribayashi) Drechs. and *C. kusanoi* (Nishikado) Drechs. respectively. The ascigerous stage of *Helminthosporium stenospilum* Drechs. on *Saccharum officinale* belongs to the helicoid series and has been named *Cochliobolus stenospilus* by Matsumoto and Yamamoto [1936]. Our fungus, both in its conidial and perfect stages, provides a close parallelism with these six species. There is a marked similarity between the perithecia of these species and our fungus. They are globose or flasked shaped, black or blackish brown in colour and have a thick pseudo-parenchymatous fragile wall; the beak of the

perithecium is without setae as in *C. miyabeanus* and *C. heterostrophus*; judging from the illustration given by Matsumoto and Yamamoto [1936] the perithecium of *C. stenospilus* also seems to be without setae, the is glabrous or may occasionally bear sterile hyphae. The perithecium may be with or without a beak. There is a considerable difference in the size of perithecia of these seven species (Table I).

TABLE I
Size of perithecia and their ostiolar beaks

	Perithecia	Ostiolar beak
<i>C. miyabeanus</i>	370—760 × 370—780 μ	95—200 × 55—100 μ
<i>C. sativus</i>	770—530 × 340—470 μ	90—150 × 80—100 μ
<i>C. setariae</i>	240—500 × 220—315 μ	60—125 × 50—100 μ
<i>C. heterostrophus</i>	400 × 400—600 μ	150 × 150 μ
<i>C. kusanoi</i>	300—350 × 300—350 μ
<i>C. stenospilus</i>	266—462 × 238—448 μ
<i>C. n. sp.</i>	220—597 × 172—406 μ	53—236 × 56—100 μ

The asci have a general resemblance in size; the range of variation both in the length and breadth of the asci of our fungus is much greater than that of the other species (Table II).

TABLE II
Size of asci and ascospores

	Asci		Ascospores		
	Length	Width	Length	Width	Septation
<i>C. miyabeanus</i>	142—235 μ	21—36 μ	235—468 μ	6—9 μ	6—8
<i>C. sativus</i>	110—220 μ	32—45 μ	160—360 μ	6—9 μ	6—8
<i>C. setariae</i>	130—150 μ	22—32 μ	200—315 μ	6—7 μ	5—6
<i>C. heterostrophus</i>	160—180 μ	24—28 μ	130—340 μ	6—7 μ	..
<i>C. kusanoi</i>	130—170 μ	14—18 μ	140—170 μ	5 μ	6—8
<i>C. stenospilus</i>	127—195 μ	20—33 μ	130—300 μ	6—8 μ	4—6
<i>C. n. sp.</i>	80—228 μ	13—34 μ	125—301 μ	4—8 μ	4—6

the smallest ascus of our fungus is much smaller than the smallest of the species; but the maximum measurement is very close to that of *maizeanus* and *C. sativus*. In width the smallest measurement is practically the same as that of *C. kusanoi* and the maximum is very close to that of *maizeanus*, *C. setariae* and *C. stenospilus*.

The number of ascospores in an ascus our fungus resembles *C. kusanoi*; we have invariably eight ascospores. In *C. heterosporus* the number varies from one to four (typically 4) and in the remaining four species it varies from eight.

The range of variation in the length of the ascospores of our fungus is exactly the same as that of *C. stenospilus*; the ascospores of the other species except those of *C. kusanoi* are longer than our fungus. There is no difference in the width of the ascospores of these species; the number of ascospores of the ascospores of our fungus is the same as that of *C. stenospilus*. The ascospores of both are mostly flagelliform.

The conidia are brown or brownish in colour fusiform or long elliptical in shape, occasionally slightly curved, five to nine septate measuring $45.6-111.4-152.2\mu$; they have never been observed to be forked; their wall is thin; hilum is present. The germination is bipolar, germ tubes have not been observed to arise from the intermediate cells.

Our fungus does not possess complete similarity with any one of the known species of the helicoid series of the ascigerous stage of the graminicolous Helminthoglyphus; though in some individual characters it may resemble one or more of these known species. Our fungus is, therefore, considered to be a new species of the helicoid series. It is congeneric with *Cochliobolus* and the binomial *Cochliobolus tritici* sp. n. is proposed.

Cochliobolus tritici sp. n. Dastur

Perithecia scattered or gregarious, black or brownish black, pseudoparenchymatous, fragile, flask shaped, with or without ostiolar beak; bodies globose, $220-597 \times 172-406\mu$; usually glabrous, at times covered with minute hyphae; beaks, when present, well developed, cylindrical, $36 \times 53-124\mu$; asci numerous cylindrical or clavate, straight or slightly curved widest below the middle, rounded at the apex; shortly stipitate at the base or sessile hyaline and thin walled $80-228 \times 13.0-34.0\mu$. Paraphyses numerous, hyaline, at times dichotomously branched, extremely fine, up to 10μ wide, septate. Ascospores, eight in number, disposed in a strongly spiral arrangement, flagelliform or filiform, obtusely pointed at the apex and slightly pointed at the base; wider at the apical portion than the basal which is tapering; four to twelve septate hyaline in colour $125.4-301.6$ by $47-76\mu$. Conidia straight or slightly curved, elliptical with broadly rounded ends five to nine septate; basal scar present, wall firm light brown to honey coloured, 45.6 to 83.6 by 11.4 to 15.2μ ; germination bi-polar. Found on kernel of *Triticum vulgare*.

Cochliobolus tritici, sp. nova

Perithecia dispersa vel aggregata, nigra vel brunneo-nigra, pseudoparenchymatica, fragilia, amphorae similia, ostiolari rostro praesente vel absente; sporangia globosa, $220-597 \times 172-406\mu$; generatim glabra, non raro tamen

operta hyphis vegetativis; rostra, si adsunt, bene evoluta, cylindrici 53—236 \times 53—124 μ ; asci plures cylindrici vel clavati, recti vel leviter curvati, latiores sub medio, rotundi in apice; sessiles vel breviter stipitati in hyalinis, et tenuibus parietibus praediti, magnitudinis 80—228 \times 13.0—30.0 μ . Paraphyses plures, hyalinae, non raro dichotome ramificatae, admodum longae, latitudinis ad 1.66 μ , septatae. Ascosporae numero 8, dispositae valde helicoidali ordinatae, flagelliformes vel filiformes, obtusae in apice, acutae in basi; latiores ad apicem quam ad tenuescentem basim; septatae, colore hyalinae, magnitudinis 125.4—301.6 \times 3.8—7.6 μ . Conidia recta vel leviter curvata, elliptica, extremitatibus late globata, 5—9 septicula, cicatrix basalis adest; parietes firmi, colore ex tenuiter brunneo ad medium praediti, 45.6—83.6 \times 11.4—15.2 μ ; germinatio bipolaris.

Habitat in seminibus *Tritici vulgaris*.

Type specimens are deposited in the herbaria of the Mycologist and Government of the Central Provinces and Berar and of the Imperial Mycologist, New Delhi.

This fungus was isolated some years back from 'black point' affected wheat kernels. During the writer's absence on leave most of the cultures of the fungus isolated from this source were lost as a result of a bad infection with mites. Since then innumerable 'black point' affected wheat kernels have been planted on agar media and moist filter papers but from none of these plantings this fungus has been obtained, though various other fungi previously secured have been re-isolated. Several methods for the surface sterilization of wheat have been adopted including the use of chemicals such as silver nitrate which Duggan [1935] has found to be less toxic than mercuric chloride.

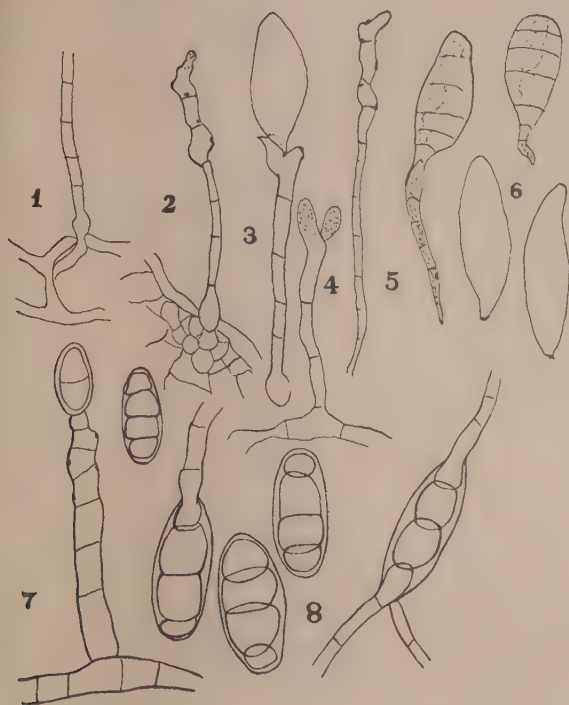
Helminthosporium sp. A

The growth of this fungus on the infected seed is characteristic; it can be readily differentiated from that of other fungi growing on 'black point' affected wheat kernels when incubated under moist conditions. The aerial mycelium is scanty, both on the pericarp of the kernel and on the filter paper on which the kernel is planted. Both the pericarp and the filter paper are covered with a black or sooty powdery mass the conidia and conidiophores.

Conidiophores

Conidiophores on the kernel are developed in two ways; either directly from the mycelium in the cells of the host tissue or from the surface layer of aerial mycelium developed on the outside of the pericarp. The mycelium in the pericarp generally forms a stroma from which the conidiophore arises; but the conidiophore may also arise directly from a hypha in the epidermal cells. When the conidiophores arise directly from the mycelium in the tissues of the kernel, the lower end of the conidiophore is swollen or bulbous (Figs. 1-3). But when the conidiophore is borne on the aerial mycelium it is a prolongation of a hyphal branch (Figs. 4 and 5); the beginning of the development of this conidiophore is marked by slight thickening, swelling and colouring of the terminal cell of this hypha. The conidium is unbranched; it usually arises from the substratum singly and is coloured light brown. The number of conidia borne on conidiophores arising from the pericarp of the kernel is small, one to seven, but of those borne on conidiophores in culture as much as 17 judging from the number of scars and geniculations present.

phores. The development of conidia is typical of the genus *Helminthium*. The conidium is borne terminally; just below the point of its attachment the conidiophore grows onward forming a geniculation and bears a conidium. The process continues making the conidiophore geniculate; the number of bends correspond with the number of conidia developed.



Helminthosporium sp. A. ($\times 270$)

- FIG. 1. A conidiophore emerging through an epidermal cell
 FIG. 2. A conidiophore developing from a stroma
 FIG. 3. A conidiophore with a conidium
 FIG. 4. A conidiophore arising laterally from a hypha
 FIG. 5. A hypha developing into a conidiophore
 FIG. 6. Conidia, two conidia are germinating

Helminthosporium sp. B. ($\times 600$)

- FIG. 7. A conidiophore developing laterally from a hypha
 FIG. 8. Conidia, two conidia are germinating

The conidiophores borne on the pericarp measure $41-243\mu$ up to the first geniculation; their bulbous ends are 5.5 to 11μ in width. The number of conidia from the bulbous end up to the first scar varies from three to six.

Conidia

The conidia both on the wheat kernel and on culture vary in shape ; they are obpyriform, obovate spear-head shaped or elongated elliptical ; the apex is either broadly rounded or pointed ; there are variations between these two extremes ; the conidia are stipitate and have a conspicuous hilum ; the stipe is about $3-9\mu$ long ; they are straight and regular but sometimes are crushed out of shape as they are crowded together ; the mature conidia are dark brown or honey coloured ; the septa are indistinct (Plate X fig. 13) ; they vary from three to seven ; at the basal end there is a distinct hyaline or lighter coloured area ; the wall is firm.

The germination is invariably from the basal end or the apical end (Fig. 6). The conidia measure $45.6-91.0 \times 18.7-30.0\mu$ germinating $52.0-78.0 \times 18.7-30.4\mu$.

In culture media the growth of this *Helminthosporium* is as characteristic as on the 'black-point' kernel. The aerial growth is limited ; the mycelium forms a thin felt-like growth ; when the conidia develop the colour becomes greenish black.

Inoculations of wheat seedlings failed to produce lesions on the leaves or stem ; but when immature ear heads were inoculated the glumes developed lesions and the kernels the typical smudge on the embryo end.

Helminthosporium sp. B.

This *Helminthosporium* differs from the other *Helminthosporia* isolated from 'black point' affected wheat kernels in the conidiophores and conidia being very small, and in the conidia having a constant number of segments, namely three.

The conidiophores are very sparsely developed directly from the tissue of the kernel ; they emerge singly between the epidermal cells of the pericarp ; they are scattered ; their basal segment is not swollen. The incubated kernel on its outside is covered by a layer of brown mycelium from which the conidiophores are developed in large numbers. They are generally borne laterally but in some cases they are borne terminally, the brown hypha bearing a conidium at its apex. The conidiophores that emerge from the host tissue or are borne laterally from the hyphae of the mycelial felt are slender, unbranched, light to dark honey coloured ; the head bearing the conidium is very slightly broader than the rest of the conidiophore ; it is not strongly enlarged at the points of attachment of the conidia ; the scars marking the points are close to each other (Fig. 7) ; up to the first scar the conidiophore has three to five septa and measure $23.0-53.2 \times 3.5\mu$; the number of conidia borne is small, about two to six.

The conidia are elliptical in shape, both ends are similar and broadly rounded ; the basal end is distinguished by a not too prominent hilum ; conidial wall is smooth, firm and thick, light to dark honey coloured ; there are three and clearly visible ; the germination is from each end, never from intermediate cells ; usually it is from the hilum end that the germ-tube develops ; only one germ-tube is developed from each end ; but from the apical end of the lowermost cell a side branch is often developed (Fig. 8). The conidia measure $18.75-30.0 \times 7.5-11.25\mu$.

This *Helminthosporium* is not the same as *H. triseptum* Drechs. isolated from velvet grass, *Notholcus lanatus* by Drechsler [1923]. *H. triseptum*

conidia, $35-50 \times 15-21\mu$; they are dark olivaceous in colour and arise only from the basal end.

The coleoptile and stems of seedlings inoculated with this fungus develop brown lesions; the roots turn brown. 'Black point' affected kernels developed from inoculated flower heads.

Phoma sp.

This disease is first noticed on wheat ears. The infected immature heads are darker green in colour than the healthy heads; this difference in colour is noticeable only when the ear is green; when it matures and turns brown the colour of the infected ear is the same as that of the healthy ear. A few or all the spikelets in an ear may be affected. The infection is first seen on the outer surface of the glume; it commences as a minute pale brownish speck; it enlarges elliptically along the length of the glume forming a diffused lesion and ultimately may cover the greater part of the glume; the diseased area later turns tobacco brown; at an advanced stage the central part of the lesion turns lighter in colour, pale straw coloured or silvery grey coloured; the lesion thus has a distinct dark brown border, the outline of which is not sharply defined. In the pale coloured centre pycnidia are developed; they are not scattered but are arranged in rows along the vascular strands or veins. The infection may spread to the inner surface of the glume; the lesion is diffused and tobacco brown in colour; pycnidia in the inner rows are developed on the inner glume as well. The kernel may be shrivelled or aborted. The infected kernel does not necessarily show a black smudge at the embryo end; the lesion is usually a brown line along the furrow, other parts of the pericarp may also be affected.

In transverse sections of a glume or a pericarp through a lesion with pycnidia it is seen that they may originate in cells just below the epidermis, so that the pycnidium looks as if it had developed superficially, or they may originate in the inner tissues, in which case the mature pycnidium fills practically the whole thickness of the glume or the pericarp (Plate XXIX, figs. 10 and 11). In most tissues the pycnidia do not seem to be embedded in a stroma (Plate XXIX, figs. 10 and 11). The pycnidia burst through the epidermis. When 'black point' affected kernels are planted on moist filter paper and incubated in glass chambers under aseptic conditions at room temperature the fungus does not develop a prominent growth of the aerial mycelium; it is scanty and spreads out fan-like on the filter paper; the colour is brownish or blackish. On the filter paper the pycnidia are formed singly, are scattered and isolated; there is no trace of the presence of a stroma. On the incubated kernels also the mycelial growth is very scanty and appressed to the pericarp. Pycnidia may be crowded together but there is no development of a stroma. The pycnidia also pycnidia are without a stroma. They are thick walled, hyaline to carbonous, pear shaped or sub-globose, and bear a short but distinct beak (Plate XXIX, fig. 12). When seen from above under high magnification a distinct ostiole or opening is visible; the ostiole is not minute; the beaks are absent or obsolete; the pycnidia measure $38-53 \times 152-228\mu$. Pycnidia escape from the ostiole in a long tendril or worm-like mass. They are hyaline elliptical or ovoid, one celled and non-guttulate. They measure $6.7 \times 1.7-3.0\mu$; conidia when placed in water swell considerably and

become bi-cellular before they germinate. Germ tubes are developed both ends.

Wheat seedlings and ears inoculated with this fungus gave positive results. Brownish to blackish elongated lesions were formed on the stem ; on the leaves typical diffused elongated tobacco coloured lesions develop ; on the glumes enclosed by the inoculated glumes the embryo end was discoloured and sometimes there were also lesions on the pericarp.

TAXONOMY

Our fungus belongs to the family *Phomaceae*, sub-family *Hyalosporaceae*. In this sub-family, it is very near the genus *Phoma* Fr., em Desm. According to Saccardo [1884] in *Phoma* the pycnidia are not beaked, the ostiole is rudimentary or obsolete, and the spores are mostly two-guttulate. As our fungus has these characteristics it is doubtful if it can be placed in this genus. The important difference between this genus and *Pseudophoma* v. Hoehn., according to Clements and Shear [1931], is that the latter has rostrate pycnidia and the spores are hystogenic. Von Hoehnel [1916] gives the following description of the new genus created by him :—'Stromata sub-epidermal ganz pycnidial, mit allseitig gleichmässig entwickelter, gut abgegrenzter Kruste mit schnabelartigem Fortsatz, der (allein) nach aussen durchbricht. Jedes eine aus einer Gewebszelle des Stromainnern histolytisch entstehend, keulenförmig, stabchenartig, ziemlich gross, durch den schliesslich oben ausbrechenden Schnabelfortsatz entleert.'* On wheat glumes and kernels thick-walled bodies, brown to black in colour are developed in the tissues of the host, completely or partially filling the thickness of the glume or the pericarp ; whereas in some cases at least, these pycnidial bodies are 'stromata ganz pycnidial', it is difficult to say ; but in cultures, on agar media wheat stems, bran and on moist filter papers, there is no stromatic development ; shaped or globular thick-walled pycnidia with a short but distinct beak developed, singly or in clusters ; the beak is clearly ostiolate. The conidia do not seem to be developed by hystolysis ; the basidia are obsolete.

As our fungus has rostrate pycnidia with a distinct ostiole it is provisionally placed in the genus *Pseudophoma* v. Hoehn., even though it does not wholly answer to the description given by v. Hoehnel.

Pseudophoma sp.

Pycnidia sub-epidermal, without stromata, thick-walled, coriaceous to carbonous, pear shaped or sub-globose, with a short beak, ostiole 38—53 × 152—228 μ ; basidia obsolete ; conidia hyaline, elliptical or oblong, one-celled, non-guttulate, escaping through the ostiole in long tails 5.0—6.7 × 1.7—3.0 μ ; germination bi-polar ; when placed in water they swell and become bi-cellular.

Nigrospora sphaerica (Sacc.) Mason

A *Nigrospora* was isolated both from the 'black point' affected wheat kernels and from spotted glumes of rice (*Oryza sativa*).

*My thanks are due to Dr G. W. Padwick, Imperial Mycologist, and Dr Mundkur, Assistant Imperial Mycologist, Imperial Agricultural Research Institute, Delhi, for very kindly supplying me the original descriptions of *Phoma* and *Pseudophoma*.

The growth of the fungus on wheat kernels incubated on moist filter under aseptie conditions is white and sparse ; later the mycelium is appressed to the surface of the kernel and the filter paper ; it does not form a compact mass ; the white colour is soon replaced by a diffused black or ash colour—a sort of pepper and salt colour—on account of the development of conidia bearing hyphae which are brown in colour. The conidia are singly on short swollen or vesicular basidia, which may be hyaline or brown in colour. The conidia are broadly elliptical with rounded ends or are subglobose, when seen from the side ; they are round when seen from the top ; they are deep, dark brown or black in colour and opaque ; under magnification they are seen to have a central lighter coloured globular nucleus. The short diameter of the conidium is in continuation of the vertical axis of the basidium. The conidia borne on the mycelium originating from the infected wheat kernels are smaller than those produced in cultures. The conidia measure $13.0-19.0 \times 9.5-15.0 \mu$; those developed on rice meal agar measure $15.0-26.0 \times 11.0-18.7 \mu$. The *Nigrospora* isolated from rice glumes is similar to that isolated from wheat kernels.

Miyake [1910] has described *Epicoccum hyalopes* Miyake on rice glumes in which to Mason [1927], from the description given, ' seems undoubtedly *Nigrospora* '. As the conidia measure $14-18 \times 13-15 \mu$, Mason considered it to be *N. sphaerica* (Sacc.) Mason. Palm [1918] has described *Nigrospora sphaerica* Palm from wheat glumes. The conidia measure $22-30 \mu$; they are much bigger than those developed by the fungus isolated from wheat and rice glumes. This fungus is, therefore, considered to be *Nigrospora sphaerica* Mason.

Inoculations of seedlings and ears of wheat and rice isolated from these two sources have given negative results.

Uromyces rolfii Sacc.

Numerable ' black point ' affected kernels have been incubated under aseptie conditions ; but this sclerotial fungus, was isolated only from half a dozen kernels.

This fungus was identical with that isolated from roots of wilted wheat and from other hosts such as tomatoes, potatoes etc. It is, therefore, not necessary to give a detailed description of the fungus.

Inoculations of wheat seedlings and green ears were successful.

Uromyces sp.

This fungus has often been isolated from ' black point ' wheat kernels and rotted glumes of rice (*Oryza sativum*).

On rice glumes at first a brownish speck is visible ; this increases in size and ultimately the centre of the lesion turns white and the margins become brownish black. The white or the central part of the lesion is dry and depressed, it ultimately cracks ; minute black sclerotia-like bodies are visible even with the naked eye in the white part of the lesion. The lesion is confined only to the glume ; the rice grain or seed is normal.

The sclerotia are globose and black in colour ; they measure $23-61 \mu$.

Inoculations of wheat and rice seedlings and ears were unsuccessful.

SUMMARY

An account of some fungi not previously recorded on 'black point' affected wheat kernels is given. The fungi described are *Cochliobolus* n. sp., *Helminthosporium* sp. A. and *H.* sp. B., *Pseudophoma* sp., *Nigrospora sphaerica* (Sacc.) Mason and *Rhizoctonia* sp. *Sclerotium rolfsii* Sacc. have been isolated from 'black point' affected kernels.

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EXPLANATION OF PLATE XXIX

Cochliobolus tritici n. sp.

- FIG. 1. Section of a wheat pericarp through a perithecium
 FIGS. 2 & 3. Perithecia with and without beaks
 FIG. 4. Asci with paraphyses
 FIG. 5. A group of asci
 FIG. 6. A stipitate ascus
 FIG. 7. A non-stipitate ascus
 FIG. 8. Asci showing the helicoid arrangement of ascospores
 FIG. 9. Ascospores

Pseudophoma sp.

- FIG. 10. Section of a wheat pericarp through pycnidia
 FIG. 11. Section of a wheat glume through pycnidia
 FIG. 12. Pycnidia with beaks

Helminthosporium sp. A

- FIG. 13. A group of conidia

THE FIXATION OF ELEMENTARY NITROGEN BY SOME OF THE COMMONEST BLUE-GREEN ALGAE FROM THE PADDY FIELD SOILS OF THE UNITED PROVINCES AND BIHAR

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INTRODUCTION

At the present time, evidence has been adduced to show that one of the probable causes for the preservation of the fertility of the paddy field is through the fixation of atmospheric nitrogen. In this connection [1929] has demonstrated the presence of a nitrogen-fixing bacterium in the root of the rice plant, after the manner of the Leguminosae, while [1932] has obtained indications that the rice plant itself possesses the power of assimilating elementary nitrogen. De [1936], working with a mixed culture of algae and bacteria, came to the conclusion that the fixation of nitrogen in rice soils under water-logged conditions is an algal process, while the absence of fixation in the cultures kept in the dark implies that bacteria cannot alone be involved. De and Bose [1938] found that in water-logged period conditions are unfavourable for certain bacteria *Azotobacter* which are unlikely to be very active at this time. By far the most important works on this subject have been those of Fritsch and De [1938] and De [1939]. They have concluded that nitrogen-fixation in these soils is purely through the agencies of algae and the part played by bacteria is relatively unimportant and possibly nil. They further found that nitrogen-fixation was confined to species of *Anabaena*, while *Phormidium foveolatum* afforded no evidence of fixation. Lately Uppal, Patel and Daji [1939] have shown that *Azotobacter* plays an important rôle in the nitrogen recuperation of rice soils at Karjat.

The present work arose out of the observation that in the paddy fields of the United Provinces and Bihar there is an universal growth of a plant community constituted mainly by *Aulosira fertilissima* Ghose intermingled with filaments of *Anabaena ambigua* Rao, *Anabaena fertilissima* and *Cylindrospermum gorakhporensis* Singh. This association forms a thick and compact stratum, and sometimes it becomes so extensive as to cover the surface of a field completely, interrupted only at places where paddy plants grow out. At the close of the harvest period the above blue-greens are observed to be reproducing freely by spores. After a fortnight or so it was found that the plants disintegrated and died, leaving behind only spores for perennation. The presence of a large number of spores in the

upper layers of the soil was availed of, and it was thought desirable to start cultures with these spores because of the little chances of contamination from bacteria and fungi.

For the sake of comparison *Protosiphon botryoides* (Kütz.) Klebs *parieticola* Iyengar, a member of the Chlorophyceae, was isolated from a paddy field of the Benares district. It was observed that the vesicles of the alga contained a number of thick-walled cysts, some of which were also found in the soil. After a week the plants died leaving behind only the roots. The cultures in the present case were, therefore, started with the cysts.

CULTURE METHODS AND ISOLATION OF THE ALGAE

The culture solution used for the growth of these organisms was a modification [1939] of Benecke's solution [cf. Kufferath, 1930] substituted with KNO_3 for NH_4NO_3 . Its composition is as follows: KNO_3 , 0.1 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 gm.; CaCl_2 , 0.1 gm.; KH_2PO_4 , 0.2 gm.; FeCl_3 , (1 per cent), 2 drops; water (pyrex distilled), 1,000 c.c. In some experiments sterile soil-extract was used as the basal medium; in others where the effect of changes in pH of the culture medium on growth of the algae and their nitrogen-fixing capacity was to be studied the above modification of Benecke's solution was buffered with potassium phosphates (mono-, di-, or triphosphates) to give the desired pH; while still in others where the importance of K and Ca ions on growth and activity of these plants was investigated various other modifications were used. In some cases nitrogen-free media were utilized and those generally used were: (1) modified Benecke's solution with the omission of KNO_3 ; (2) solution containing, K_2HPO_4 , 0.5 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 gm.; CaSO_4 , 0.1 gm.; FePO_4 , 0.1 gm.; $\text{Ca}_3(\text{PO}_4)_2$, 1.0 gm.; FeCl_3 (1 per cent), 2 drops and water (pyrex distilled), 1,000 c.c.

In order to obtain uni-algal cultures the following method was adopted. Soil blocks without the least disturbance of the surface layers were brought to the laboratory and examined under a dissecting microscope. This revealed a large number of spores mostly on the surface of the soils. Also, the spores of the different species of algae under consideration were found in groups, thus facilitating a good deal of their isolation, which was carried out as follows. The lumps of spores were removed from the soil with a pair of hot needles. A clean sterilized glass slide in a drop of sterilized water and under the microscope the adhering soil particles were, as far as possible, removed. New spores were transferred with hot forceps to a test tube containing a drop of sterilized distilled water and closed with a rubber stopper and shaken vigorously. The suspension was then allowed to stand for 15 minutes and the supernatant turbid liquid was decanted off. This process was repeated several times until the supernatant liquid became perfectly clear. Finally the spores and in the case of *Protosiphon botryoides* the cysts along with a few c.c. of liquid were transferred to a sterilized centrifuging tube in sterilized distilled water and centrifuged. The suspension was further diluted five times again centrifuged. A loopful of this suspension was pipetted out by means of a hot pipette and transferred to several agar plates, and these were exposed to light when after 15 or 20 days many showed good growth. Numerous filaments radiated from the points of inoculation, and single he-

es were selected and their positions marked with Indian ink under a microscope. Portions of the agar including such marked areas were then cut out, transferred to the liquid medium mentioned previously and allowed to grow in 250 c.c. pyrex Erlenmeyer flasks.

For getting bacteria-free cultures silica gel plates were utilized. The silica gel was prepared by mixing equal volumes of hydrochloric acid (sp. gr. 1.1) and potassium silicate solution (sp. gr. 1.06). Merck's sodium silicate pure crystals were used and the solution was made up with cold water. In a number of 9 cm. Petri dishes, 40 c.c. portions of the mixture were placed and after 48 hours when the gel had hardened, the plates were first washed with running tap water until free from acid and subsequently several times with boiled distilled water. Each plate was then impregnated with 5 c.c. of the following solution: KNO_3 , 0.1 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 gm.; K_2HPO_4 , 0.1 gm.; CaCl_2 , 0.1 gm.; FeCl_3 (1 per cent), 1 drop; water (pyrex distilled), 100 c.c. Finally the plates were exposed to a temperature of 60°C . until the surface of the gel was fairly dry and then sterilized in an autoclave at a pressure of one and a half atmosphere for 20 minutes.

A loopful of the centrifuged suspension containing at the most five spores or cysts on the average was pipetted out and transferred by means of a hot sterilized pipette to the centre of a sterilized silica gel plate and spread over the surface by means of a hot glass rod. After this the plates were exposed to diffused light obtained from a north window in the laboratory. It took about a month or so for the spores to germinate and form algal filaments and in the case of the cysts the time taken was a little longer. After the appearance of the filaments a little of each was transferred to one of the soil-extract-nitrate-cane-sugar-agar plates described below, and, if found uncontaminated, fresh sub-cultures on silica gel were made as above and the process repeated. When one or more colonies were obtained, which appeared to be pure when tested by the above method, they were transferred to a culture medium and allowed to grow for sometime. They were finally tested for purity by the methods described below. In this way *Aulosira fertilissima*, *Cylindrocapsa gorakhporensis*, *Anabaena ambigua*, *A. fertilissima* and *Protosiphon obovatus* forma *parieticola* were obtained in pure cultures.

The following media, both solid and liquid, were used for testing the purity of the cultures: (1) nutrient agar, (2) soil-extract-nitrate-cane-sugar-agar (De's modification of Benecke's solution given above with 50 c.c. soil-extract and 15 gm. cane-sugar), (3) Beijerinck's medium containing 20 gm. mannite, 0.2 gm. K_2HPO_4 , and 1,000 c.c. tap water, (4) medium containing 1 per cent mannitol, 0.2 gm. K_2HPO_4 in 1,000 c.c., 10 gm. CaCO_3 in 1,000 c.c. and 1,000 c.c. tap water. Several drops of a suspension of the supposedly pure algal growth were inoculated into the above media, which were then incubated for a week in the dark at 25°C . the presence or absence of turbidity and growth of bacterial colonies along the line of inoculation in liquid and solid media respectively, being taken as an index of the presence or absence of bacteria.

The isolation from bacteria by growing the algae on sterilized silica gel plates was also started with the uni-algal filaments but it was observed that none of the cultures produced turbidity when grown in the testing media.

It is, therefore, concluded that it is safer to start with spores in order to obtain pure cultures.

Nitrogen analyses were made by the macro-Kjeldahl method. At the end of an experiment the entire contents of the culture vessel (medium and alga) were poured into a Kjeldahl flask, any growth adhering to the sides of the vessel being washed out with pure conc. sulphuric acid. Total nitrogen was estimated by the Gunning-Hillbald modification of Kjeldahl's method [Wright, 1939]. Digestion and distillation were carried out in the usual way, the ammonia evolved being absorbed in $N/15$ H_2SO_4 and estimated by back titration with $N/15$ $NaOH$, using methyl red as an indicator. For the determination of small amounts of nitrogen, $N/50$ acid and alkali were used and the acid boiled to drive off CO_2 before titration.

Pyrex Erlenmeyer flasks of 250 c.c. capacity were used for growing the cultures and the mouth of each flask was plugged with cotton-wool. A dozen empty flasks were, at one time, sterilized in an autoclave at a pressure of one and a half atmosphere for 20 minutes. After 24 hours the flasks were subjected to a second heating under the same pressure to ensure the complete elimination of bacteria. Next in each one of these flasks 250 c.c. of the culture medium was kept, the plugs replaced, and the flasks again heated twice after the manner described above. The flasks were then allowed to stand for two days before being inoculated with a suspension of the alga in water. All cultures were grown under laboratory conditions of temperature and pressure. An overhead Philips electric lamp of 250 v. provided illumination of constant intensity adjusted to such a height that it did not affect the temperature of the surroundings of the cultures. On an average the cultures were illuminated for 10 hours daily.

NITROGEN-FIXATION BY THE ALGAE

Five replicates were made for each treatment and the experiments were conducted in two series. The mean of the replicates and ultimately the mean of the two series has been taken as the criterion of the nitrogen-fixing capacity of the algae in question. The results are presented in Tables I-IV.

1. *Aulosira fertilissima* Ghose. In both the series in all cases the growth of the alga in the beginning was quite rapid and it formed a membranous blue-green stratum on the surface of the culture medium, which in most of the flasks extended on the sides above the level of the liquid, irrespective of the medium containing nitrate, or not. After a week's incubation the algal stratum, in cultures with nitrate in the medium and especially in those having soil-extract as the basal medium, began to fade out and finally became pale or almost colourless; while in cultures without nitrate, it continued to retain its bluish-green tinge even after the second week. At the end of the second week, however, in some of the flasks, with nitrate and soil-extract, patches of green colour began to appear on the surface, signifying thereby the renewed growth of the alga. The algal stratum, however, never became so thick and compact as that found in natural conditions. After the third week's incubation the behaviour of the cultures as described above remained similar and it continued to be so even after the close of the experiment.

TABLE I

Nitrogen-fixation by Aulosira fertilissima Ghose

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series			
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate	Mean of series
(De's modification of Be-solution KNO_3)	...	7.3	7.3	7.4	7.7	7.7	7.6	7.5
		7.6	7.6		7.4	7.4		
		7.2	7.2		7.6	7.6		
		7.4	7.4		7.5	7.5		
		7.5	7.5		7.8	7.8		
+ soil-ex-	0.4	8.4	8.0	8.08	8.6	8.2	8.02	8.05
		8.5	8.1		8.3	7.9		
		8.5	8.1		8.5	8.1		
		8.6	8.2		8.3	7.9		
		8.4	8.0		8.4	8.0		
+ nitrate	2.0	5.8	2.9	2.02	6.0	3.1	2.68	2.8
		6.2	3.3		5.5	2.6		
		5.6	2.7		5.3	2.4		
		5.9	3.0		5.7	2.8		
		5.6	2.7		5.4	2.5		
+ nitrate extract	3.3	11.8	8.5	8.8	11.7	8.4	8.6	8.7
		12.2	8.9		11.6	8.3		
		12.4	9.1		12.0	8.7		
		12.0	8.7		11.9	8.6		
		12.1	8.8		12.3	9.0		

Cylindrospermum gorakhporens Singh. The growth of *Cylindrospermum gorakhporens* was always submerged and it formed a thick dull-green irregular mat on the bottom of the flask. In soil-extract-nitrate medium the growth was slow in the beginning and it was not until the commencement of the second week that the algal stratum became visible at the bottom of the flask. In free-soil-extract and N-free-nitrate media, however, the growth was rapid at the start but after a week's incubation the stratum became thin and finally colourless. It renewed growth after the second week's incubation. On the whole, in the latter two media, the algal cells were more fully developed. The heterocysts were very much elongated and the contents, which were at first granular became homogenous and pale

Anabaena ambigua Rao. The growth in case of this alga was, in the beginning, submerged, the colonies being in the form of narrow cylinders, but later, almost erect. Later, however, they had the tendency of rounding

up and coming up on the surface of the culture medium. In the N-extract and N-free-nitrate media the growth was quick at the start, after two weeks' incubation the colonies began to lose colour and effluent, finally getting mixed up with the medium. In the soil-nitrate medium, however, the algal colonies were quite intact till the end of the experiment.

TABLE II

*Nitrogen-fixation by *Cylindrospermum gorakhporens* Singh*
(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series		
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate
1. N-free (De's modification of Bencke's solution without KNO_3)	...	4.5	4.5	4.28	4.1	4.1	4.02
		4.2	4.2		3.8	3.8	
		3.8	3.8		3.9	3.9	
		4.6	4.6		4.2	4.2	
		4.3	4.3		4.1	4.1	
2. N-free + soil-extract	0.4	4.8	4.4	4.68	5.4	5.0	4.78
		5.2	4.8		5.5	5.1	
		5.3	4.9		4.9	4.5	
		5.2	4.8		4.9	4.5	
		4.9	4.5		5.2	4.8	
3. N-free + nitrate	2.9	4.9	2.0	2.46	4.8	1.9	2.3
		5.5	2.6		4.7	1.8	
		5.6	2.7		5.4	2.5	
		5.4	2.5		5.6	2.7	
		5.4	2.5		5.5	2.6	
4. N-free + nitrate + soil-extract	3.3	8.3	5.0	4.82	8.6	5.3	5.0
		7.9	4.6		8.3	5.0	
		7.8	4.5		8.2	4.9	
		8.2	4.9		8.0	4.7	
		8.4	5.1		8.4	5.1	

4. *Anabaena fertilissima* Rao. The growth, in this case, consisted of spherical colonies of blue-green colour at the bottom of the flask. After a week's incubation aggregated to form irregular bigger colonies. The colour changed to brownish-black. In the N-free soil-extract and free nitrate media the growth of the alga was quite quick and it remained so till the close of the experiment. In the N-free and N-free nitrate extract media the growth was slow in the beginning but after the third incubation it became quite vigorous,

TABLE III

Nitrogen-fixation by Anabaena ambigua Rao

Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series			
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate	Mean of series
De's modification of Be-solution (KNO ₃)	...	3.8	3.8	3.6	3.5	3.5	3.56	3.58
		3.6	3.6		3.4	3.4		
		3.3	3.3		3.7	3.7		
		3.7	3.7		3.6	3.6		
		3.6	3.6		3.6	3.6		
+soil-ex.	0.4	4.2	3.8	4.06	4.7	4.3	4.22	4.14
		4.6	4.2		4.5	4.1		
		4.5	4.1		4.6	4.2		
		4.6	4.2		4.6	4.2		
		4.4	4.0		4.7	4.3		
-nitrate	2.9	5.0	2.1	2.26	5.3	2.4	2.3	2.28
		5.4	2.5		5.5	2.6		
		4.8	1.9		5.4	2.5		
		5.2	2.3		4.9	2.0		
		5.4	2.5		4.9	2.0		
+nitrate extract	3.3	9.3	6.0	5.58	8.6	5.3	5.66	5.62
		9.2	5.9		8.9	5.6		
		8.6	5.3		8.7	5.4		
		8.7	5.4		9.4	6.1		
		8.6	5.3		9.2	5.9		

Protosiphon Botryoides (Kütz.) Klebs forma *parieticola* Iyeng. The growth of the present alga was completely retarded in N-free and N-free-soil-extract media. There was slight growth in the beginning in the N-free-nitrate medium but after the third week's incubation a few vesicles of the plant were found attached to the sides of the flask above the culture medium. In N-free-nitrate-soil-extract medium, however, the growth was retarded, and clusters of dark-green vesicles appeared at the bottom and the sides of the flask. There was, however, no increase in the nitrogen content, which meant that the alga was unable to fix nitrogen from the atmosphere. During the fourth week's incubation in the last medium the vesicles began to migrate fast and within four days the plant died completely, perhaps due to the deficiency in the nitrogen content of the medium. The same retardation was recorded in regard to the N-free-nitrate medium, as the growth was completely inhibited after the fourth week's incubation.

TABLE IV

Nitrogen-fixation by Anabaena fertilissima Rao

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Media	First series				Second series		
	Initial N	Final N	N fixed	Mean of replicate	Final N	N fixed	Mean of replicate
1. N-free (De's modification of Be-necke's solution without KNO ₃)	...	4.6	4.6	4.6	4.7	4.7	4.64
		4.8	4.8		4.9	4.9	
		4.5	4.5		4.5	4.5	
		4.6	4.6		4.4	4.4	
		4.5	4.5		4.7	4.7	
2. N-free + soil-extract	0.4	5.7	5.3	5.3	5.5	5.1	5.14
		5.6	5.2		5.4	5.0	
		5.7	5.3		5.6	5.2	
		5.7	5.3		5.6	5.2	
		5.8	5.4		5.6	5.2	
3. N-free + nitrate	2.9	5.8	2.9	2.76	5.3	2.4	2.74
		5.9	3.0		5.8	2.9	
		5.3	2.4		5.7	2.8	
		5.6	2.7		5.6	2.7	
		5.7	2.8		5.8	2.9	
4. N-free + nitrate + soil extract	3.3	9.8	6.5	6.56	10.2	6.9	6.66
		10.0	6.7		10.0	6.7	
		9.6	6.3		10.0	6.7	
		9.7	6.4		9.8	6.5	
		10.2	6.9		9.8	6.5	

FACTORS DETERMINING GROWTH AND NITROGEN-FIXATION

1. *Illumination.* The optimum light intensity was found to depend to a marked extent upon the growth conditions, particularly, the medium. In nitrogen-free media the growth and the nitrogen-fixation capacity of *Aulosira fertilissima* Ghose were accelerated to a marked extent with increasing light intensity but the cultures could not stand the direct sunlight or high intensity for a long time as after the fifth week's incubation the cells began to disintegrate and finally disappeared completely. In diffused light, obtained from a north window, the growth proceeded slowly and was not till the end of the third week's incubation that the algal stage became visible. Later, however, the growth became greatly increased and the vigour of the cultures remained more or less constant till the close of the experiment. The nitrogen-fixation capacity also increased. These cultures were illuminated, on the average, for 10 hours daily. Some cultures were kept in the dark, and in these cases the growth appeared to be very slow and it remained so till the end of the experiment. The nitrogen-

city of the alga also remained almost constant. But, it increased very considerably in such cultures as were provided with 1 gm. of sugar per 100 of the medium. It was, however, found that the best light conditions for maximum growth and nitrogen-fixation was intermittent light, i.e., when the cultures were daily kept alternately in diffused light and direct light for five hours. In media containing nitrogen the growth was greatest in direct light, but the nitrogen-fixation capacity of the alga was slightly retarded. The reason for the latter behaviour is obvious. The results of the above mentioned experiments, carried out with *Aulosira fertilissima*, are given in Table V.

TABLE V

Growth and nitrogen-fixation by *Aulosira fertilissima* Ghose under varying light conditions

(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Illumination		Medium used	Growth	N fixed
Exposure period in hours	Light source			
10	Direct sunlight	N-free	Vigorous, retarded totally after five weeks	6.3
10	Direct sunlight	N-free + soil-extract	Vigorous, retarded totally after five weeks	6.8
10	Direct sunlight	N-free + nitrate	Best	4.2
10	Direct sunlight	N-free + soil-extract + nitrate	Best	5.3
10	Diffused light	N-free	Slow in the beginning, increased after third week and remained constant	7.8
10	Diffused light	N-free + soil-extract	Comparatively quick	6.6
10	Diffused light	N-free + nitrate	Quick in the beginning, but after second week it was retarded	2.5
10	Diffused light	N-free + nitrate + soil-extract	Quick in the beginning, but after second week it was retarded	3.8
10	Dark	N-free	Very slow but remained constant	3.5
10	Dark	N-free + 1 gm. sugar per 100 c.c. of medium	Very slow but remained constant	4.5
10	Dark	N-free + soil-extract + nitrate	Very slow and did not continue long	2.3
10	Direct light (five hours each) and diffuse	N-free	Excellent	8.6
10	Direct light (five hours each) and diffuse	N-free + soil-extract	Excellent	7.6
10	Direct light (five hours each) and diffuse	N-free + nitrate	Slow in the beginning	4.7
10	Direct light (five hours each) and diffuse	N-free + nitrate + soil-extract	Slow in the beginning	5.3

2. *Hydrogen-ion concentration.* The effect of pH on growth and nitrogen-fixation capacity was studied on *Aulosira fertilissima* Ghose. The results of the various experiments are given in Table VI. It is seen from this

table that a neutral or slightly alkaline medium is decidedly preferable for the alga. Growth could not take place below pH 6.5. It was initiated at pH 6.5 and with increasing pH it became more and more vigorous, normal being realized at 7.2. At higher pH values, although the growth to all outward appearances, was vigorous the algal cells were found to be abnormally elongated, especially the heterocysts. The nitrogen-fixing capacity of the alga also increased with increasing pH . It has also been observed that with longer period of incubation the pH of the media begins to decrease, after three weeks' incubation in case of the nitrogen-free media and after a week's incubation in media containing nitrogen. This is perhaps due to the disintegration of the algal cells.

TABLE VI

Growth and nitrogen-fixation at different pH by Aulosira fertilissima Ghiesb.
(Nitrogen in mg. per 100 c.c. of the medium. Period of incubation 45 days)

Initial pH	Relative growth (after 20 days incubation)	N fixed	Final pH
5.2 . . .	None	5.0
5.5 . . .	None	5.2
5.7 . . .	None	5.8
6.0 . . .	None	5.4
6.3 . . .	None	6.1
6.5 . . .	Slight	2.6	6.0
6.8 . . .	Fair	4.2	6.2
7.2 . . .	Normal	7.8	6.8
7.4 . . .	Vigorous	7.8	7.0
7.6 . . .	Vigorous	8.1	7.3
8.0 . . .	Abnormal (decaying) . . .	6.5	7.5
8.4 . . .	Abnormal (decaying) . . .	5.8	7.3
8.8 . . .	Abnormal (decaying) . . .	2.5	7.8

3. *Calcium and potassium ions.* It was reported by Allison, Hoover and Morris [1937] that neither calcium nor strontium, at least in concentrations greater than traces, was necessary for growth in the presence of combined nitrogen for *Nostoc muscorum* Ag. In nitrogen-free medium, nitrogen fixation, however, decreases greatly in the absence of these ions, suggesting the

they play a catalytic role in nitrogen-fixation, as in the case of *eter*. Similar experiments were conducted with *Aulosira fertilissima*. The results of these experiments are given in Table VII. These are in close agreement with those of the above mentioned workers as the behaviour of the calcium ion is concerned. It has also been found that calcium carbonate as against calcium sulphate or calcium chloride is most effective for growth and nitrogen-fixation. In another series of experiments, where basal medium consisted only of K_2HPO_4 and the culture was grown on a calcium-free medium, practically no nitrogen was obtained, except where either combined nitrogen or calcium was added. Under such conditions the nitrogen-fixation capacity of the alga also increased.

TABLE VII

Effect of Ca and K ions on growth and nitrogen-fixation in *Aulosira fertilissima* Ghose

Medium in mg. per 100 c.c. of the medium. Period of incubation 45 days. Basal medium: K_2HPO_4 , 0.75 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; $Cl_3 \cdot 6H_2O$, 0.005 gm.; H_2O , 1,000 c.c.)

Treatment	Relative growth (after 20 days incubation)	N fixed
(basal medium)	None	..
10 mg.	Slight	3.2
10 mg.	Slight	1.8
10 mg.	Normal	8.2
10 mg.	Slight	2.3
10 mg. + $CaSO_4$, 10 mg.	Normal	6.8
10 mg. + $CaCl_2$, 10 mg.	Normal	5.7
10 mg. + $CaCO_3$, 10 mg.	Vigorous	8.6

DISCUSSIONS

A summary of our knowledge of the occurrence and rôle of blue-green algae in nature would seem to indicate that these organisms are of considerable importance in the maintenance of soil fertility [cf. Bharadwaja, 1940; Stokes, 1941]. The results obtained by Allison and coworkers [1937] with the most active nitrogen-fixing blue-green alga, *Nostoc muscorum* Ag., may maintain it, lend further support to the same viewpoint. A similar conclusion was reached by Bristol [1920], even though nitrogen-fixing ability in *Cyanophyceae* had not been demonstrated conclusively at that time. Petersen [1935], however, is doubtful about the economic importance of blue-green algae in soil, basing his views largely on the supposition that they make no growth except at the soil surface. He considers that the *Myxophyceae*

with the exception of *Nostoc punctiforme* and possibly a few others, grow heterotrophically or fix nitrogen in the dark. Author's results on another active nitrogen-fixing blue-green alga, *Aulosira fertilissima* Ghose isolated from the paddy field soils of the United Provinces and Bihar together with the recent results of Allison and coworkers [1937] with *Nostoc muscorum* Ag. and that of Winter [1935], as quoted by Allison and coworkers [1937] with *Nostoc punctiforme*, definitely contradict these two ideas which are in large part as a basis for Petersen's viewpoint. Whether *Aulosira fertilissima* Ghose actually makes an appreciable growth in soil where light does not penetrate still remains to be determined, but it is at least of interest to know that it has the capacity to do so. Moreover it has been observed that *Aulosira fertilissima* not only makes an appreciable growth in the dark but its nitrogen-fixing capacity also remains fairly considerable.

If the results reported here in regard to *Aulosira fertilissima* Ghose proved to be typical, it would seem that the nitrogen-fixing blue-greens thrive best in nearly neutral or slightly alkaline soils, preferably partly shaded where moisture is abundant. *Aulosira fertilissima* also shows abundant growth in freshwaters, where the pH is very low, and sometimes in distinctly acidic soils. These studies suggest that even in acid soils it may be able to continue to multiply at the surface, because by growing together and constantly removing carbon dioxide from the soil during photosynthesis it may increase the pH locally. It has also been observed that it has the tendency to raise the pH of the medium during its growth, probably due to the liberation of organic acidic substances during the death and decay of certain of its cells. In short it can be said that the alga has a great buffering capacity. Its thin, waxy sheath also enables the organism to withstand remarkably dry conditions, as Frisch [1932, 1936] and others have pointed out with *Nostoc* and other blue-greens.

The nitrogen-fixing algae, growing near the soil surface, are unique in being able to obtain both their carbon and nitrogen from the air. This, of course, explains why they appear so soon on new volcanic soils and in other places where the soil is too poor to support most other forms of plant life.

The results of the various experiments embodied in the text bring out two important conclusions: (1) blue-green algae, apart from the species of *Nostoc* and *Anabaena*, in pure cultures free from bacteria and other microorganisms are able to utilize and fix nitrogen, (2) the green algae appear to take no part in the fixation process, though the observations have been limited and confined to only *Protosiphon botryoides* (Kütz.) Klebs forma *parvula* Iyengar. So far as the first one is concerned, it has been observed that results of carefully controlled experiments, on a comparative basis, have shown that *Aulosira fertilissima* Ghose, a very prominent participant in algal flora of the paddy fields of the United Provinces and Bihar, fixes the greatest amount of nitrogen out of the other blue-greens under consideration. The results of another series of experiments show that nitrogen-fixation capacity of *Cylindrospermum gorakhporensis* Singh, another common blue-green alga from the same localities, is by no means insignificant. The other forms that were isolated from these soils are *Anabaena ambigua* Rao and *Anabaena fertilissima* Rao, which also appear to fix considerable amount of nitro-

in the atmosphere. It is legitimate, therefore, to conclude from the above observations that the recuperation of nitrogen in the paddy field soils of India is an algal process, a view expressed also by De [1939]. It may be pointed out that the paddy field soils harbour a large number of algae [Singh, 1939]—mostly blue-greens, that are likely to play an immense role in the economy of these soils. Besides their capacity of fixing atmospheric nitrogen they are beneficial in aerating the upper layers of the submerged soils [cf. Harrison and Aiyer, 1914].

Again, the present investigation has a bearing upon the theory put forward by Dhar and coworkers [1934-36], that nitrification in tropical soils is more photochemical than bacterial, and that nitrogen-fixation is a question of energy relations because more of nitrogen is fixed in soils mixed with energy-providing materials, such as carbohydrates, celluloses and fats in sunlight or artificial light than in the dark, although the *Azotobacter* numbers in the dark are very much greater than in the light. If this hypothesis is correct, then chlorophyll-bearing plants and plant organs should fix atmospheric nitrogen. But, this is not so, as all attempts to find nitrogen-fixation by higher plants other than leguminous, since the classical work of Hellriegel and Wilth [1888], have failed. The green leaf is undoubtedly the prime source of energy on this planet, where a chain of complex chemical reactions involving energy changes and transference take place but no fixation of nitrogen. On the algal side we have the results of numerous investigations which finally show that the green algae, which by no means are less in their energy relations to the blue-greens, are unable to fix nitrogen. Kossowitsch [1894], by isolating a grass-green alga, *Cystococcus*, in pure culture, found that it could not fix nitrogen. Schramm [1914] worked with seven species of the green algae and found that in the absence of combined nitrogen no growth took place, so he concluded that these algae could not under these conditions assimilate free nitrogen. Similar results were also obtained by Kuerscher [1923] for *Chlorella*. Investigations so far done on nitrogen-fixation have alleged this function in only *Nostoc* and *Anabaena* species, though Copeland [1932] mentioned forms, such as *Oscillatoria princeps*, *Osc. formosa*, *Scenedesmus labyriinthiformis* and *Phormidium laminosum*. The present work has added two new forms possessing considerable nitrogen-fixing capacity to the existing list—*Aulosira fertilissima* Ghose and *Cylindrospermum gorakhporensense* Singh. The nitrogen-fixing capacity of these plants may perhaps be due to their peculiar metabolic activities, about which very little is yet known; and not to some simple energy relations which Dhar and his coworkers explain.

SUMMARY

The investigation deals with the nitrogen-fixation ability of some of the commonest blue-green algae—*Aulosira fertilissima* Ghose, *Cylindrospermum gorakhporensense* Singh, *Anabaena ambigua* Rao and *Anabaena fertilissima* Rao—isolated from the paddy field soils of the United Provinces and Bihar. It has been found that nitrogen recuperation in these soils is an algal process and the greatest fixation, amounting to 8.05 mg. per 100 c.c. of the N-free medium in 45 days, is obtained by *Aulosira fertilissima* Ghose.

For the sake of comparison, a grass-green alga, *Protosiphon botryoid* (Kütz.) Klebs forma *parieticola* Iyeng. was also isolated from a paddy field of Benares district and studied in the same way. It has been found that the alga does not fix nitrogen.

Factors, such as, illumination, pH, and the effect of Ca and K ions on growth and nitrogen-fixation ability of *Aulosira ferulissima* Ghose, have also been investigated.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bharadwaja, for his kind guidance and criticism throughout the course of this investigation.

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THE COLD STORAGE OF FRUITS IN THE PUNJAB

I. CITRUS FRUITS : MALTA (*CITRUS SINENSIS*) AND SANGTRA (*C. NOBILIS*)

BY

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(With Plate XXX and four text-figures)

Cold storage is absolutely indispensable for the healthy development of fruit industry and it is no exaggeration to say that, but for the existence of cold storage facilities in other countries, their fruit industries would not have survived for any length of time. The main reason for this is that most of the fruits are so easily perishable that after removal from the trees, they cannot be stored even for a few days under ordinary atmospheric temperatures. Cold storage helps in extending the period of availability of fruits and thus causes considerably the fluctuations in the prices of fruits. This enables the growers to realize reasonable price for their fruits as they do not have to dump their produce in the market at any price. They can release the fruit from the cold storage as and when required to meet the demand of the market. Consumers, on the other hand, are able to secure fruits at reasonable price for a longer period in the market.

Although the fruit industry in this country has not, so far, made any great progress, still the profitable disposal of fruit has already become an acute problem for the growers. For instance, in the Punjab, Malta oranges in January can be had at almost Re. 1 to Rs. 2 per hundred, and Sangtra orange at even 12 annas a hundred, yet after two to three months these cannot be had even five times the price of this. This is the condition in case of citrus fruits which have got a fairly good keeping quality. And worse is the situation in other fruits and vegetables which cannot be stored for even a day or two under ordinary atmospheric temperature prevailing in summer. When there is glut in the market, which is quite frequent, fruits and vegetables can be had at most dirt cheap price. Tomatoes in June can be had even at eight annas a hundred and the price easily goes up ten times after sometime.

Months of April to June are notorious in regard to the prevalence of many harmful diseases like typhoid when medical people in 99 per cent of the cases, do not recommend to patients anything else but the use of fresh fruit juices which are not available at this time of the year except those obtained from fruit stored in cold stores. In short, the cold storage can prove a boon to the growers and blessing to the consumers and it would be idle to expect sound development of fruit industry in the absence of cold storage enterprise.

The importance of this problem was realized long ago and the Fruit Specialist, Punjab, had submitted proposals several years back for the installation of cold storage plant for experimental purposes but financial stringency always

stood in the way. Fortunately, a vigorous enterprise was started about 10 years back in the form of Cold Storage Company of Northern India and their request the Imperial Council of Agricultural Research, in active co-operation of the Punjab Government, agreed to start experiments on the cold storage of fruits. The Cold Storage Company supplied a small plant of one and a half ton capacity, which was purchased later on by the Punjab Government. The Punjab Government and the Imperial Council of Agricultural Research agreed to share the other expenses of the cold storage scheme in equal proportions. As the plant had a very small capacity, only a few fruits could be experimented upon at a time. Citrus being the most important fruit of the province, naturally received special attention although some other fruits like mango, pears and grapes were also experimented upon on a small scale. This paper, however, deals with the cold storage trials of Malta and Sangtra oranges alone.

REVIEW OF LITERATURE

Cold storage of Malta orange has been a subject of thorough study in other countries like the United States of America, Australia and South Africa. The results obtained in different countries vary considerably. Young and Ramsey [1930] working on Valencia and Navel oranges found that 38°F. and 45°F. respectively were suitable temperatures for these fruits and these varieties kept well for three to four months and 3½ months respectively at above temperatures. There was little change in sugar content of juice during cold storage. At 32°F. the fruit became bitter in taste after five weeks. Ramsey [1915]—cited by Friend and Nelson [1933]—recommended the employment of temperatures considerably above 32°F., i.e. 38°-40°F. for oranges. Overholser [1930]—cited by Karmarkar [1941]—found that the temperatures of 36°-38°F. were most satisfactory. At higher temperatures the losses were heavy due to shrinkage and decay and lower temperatures caused pitting of the rind. Friend and Nelson [1932] observed that at 44°-45°F., Valencia orange could be kept very satisfactorily for long periods. Wardlaw [1933] stated that 40°F. was well suited for the storage of certain classes of citrus fruits (except limes and grapefruit). He found that the loss in weight was largely a function of size and maturity and was directly related to the area of fruit surface exposed. He also advocated the use of cellophane or other thin strong wrappers, suitably impregnated with wax or other water proofing substances instead of ordinary wrapping. Stahl and Camp [1936] found that 37.5°F. proved to be the optimum temperature for the storage of unwrapped, untreated oranges and the temperatures below this were better than temperatures above. Stahl, Camp and Fife [1936] also recommended that wrapping was better than none at all. Samis [1936] working on the gas storage of Valencia oranges remarked that the fruit stored at 32°F. and 36°F. kept better and showed no wastage as compared to fruits stored at 45°F. and 70°F. Tomkins [1937] stated that 70 per cent relative humidity reduced wastage as compared to saturated atmosphere. If ventilation was sufficiently restricted to allow the accumulation of 10 per cent carbon dioxide, wastage might be increased. Cheema, Karmarkar and Joshi [1938] found 40°F. to be the best temperature for the storage of Nagpur oranges (mandarins) and that washing with antiseptic solution was of no particular

lengthening the storage life of the fruit. Cheema and others [1939] found that Malta oranges from the Punjab kept for four months at 40°F. in good condition without any wastage. Stahl and Cain [1937] recommended 85 per cent humidity and a temperature of 37°F. with 6 per cent carbon dioxide plus 1 per cent oxygen, as the most suitable conditions for the storage of oranges. Cheema and coworkers [1938] stated that 40-42°F. was most suitable temperature for the storage of Washington Navel oranges at which these kept in good condition for 12 weeks. Storage life of Valencia Late oranges at 40°F. was 14 weeks. Tomkins [1936] working on Jaffa oranges stated that as long as by the time taken for development of 10 per cent waste, storage at 40°F. was preferable to storage at 50°F. Early season (November) fruit was more susceptible to rotting by green mould than the late season (March and April) fruit. Fiddler and Tomkins [1938] found that dipping oranges in 2 per cent sodium hydroxide was as effective as 5 per cent borax and leads to less damage to skin. One per cent borax plus one per cent sodium hydroxide were as effective as 5 per cent borax alone. Vander Plank and others [1937, 1938] observed that the effect of temperature varied with the nature of fruit stored. Temperatures 50-55°F. were beneficial for the storage of under-coloured greenish oranges. At these temperatures the fruit coloured well in storage and wastage was as low as at 39°F. The fruit stored at 50°F. for about two months was liable to become stale while at 39°F. the flavour was well maintained. Williams [1938] observed that the fruit stored in room at a temperature of 36-38°F. kept much better than that at lower or higher temperatures. Wrapped fruit was better than unwrapped fruit. Karmarkar and Joshi [1938] found that percentage loss in weight of small fruit was always greater than that of big fruit except in case of grape fruit at 68°F. Rose and others [1938] recommended the use of 32-34°F. and 80-90 per cent humidity for the storage of Washington Navel and Valencia Late oranges, at which storage life was 10-12 weeks.

MATERIAL

Two important citrus fruits, viz. Malta orange (*Citrus sinensis*) and Valencia Late (*C. nobilis*) were included in the cold storage trials during 1938 and 1939.

Malta oranges.—Five varieties of Malta orange, viz. Common, Blood Red, Valencia Late and Seville were stored during 1938-39 and 1939-40. Musambi was tried in 1940-41. Malta Common, widely cultivated, is a heavy bearer, and normally quite pleasant in taste. Blood Red is the choicest variety from the Punjab and is liked very much due to the red colour of its flesh, pleasant taste and agreeable aroma. Valencia Late is a late ripening variety, and possesses good flavour. Seville is a heavy bearer. Musambi is popular with the public as it has very little acid.

Malta Common and Blood Red were obtained from S. Mangal Singh's orchard near Shahdara, Lahore, in the beginning of March; Valencia Late from the Indian Mildura Fruit Farms Ltd. Renala Khurd, in the second week of March and Seville was obtained from the Experimental Fruit Garden at Lyallpur in both the seasons in January. Musambi was obtained from Montgomery and Lyallpur districts during the second week of January.

Sangtra oranges.—Sangtra is most commonly cultivated in the Punjab. The fruit is puffy or loose skinned and is easily damaged. The fruit is acidic (0.92 gm. citric acid per 100 c.c. juice) but when mature, is quite pleasant in taste. Two lots of Sangtra, one from Pathankote side and the other from Lyallpur, were tried during the two years of the investigations.

THE COLD STORAGE PLANT

The cold storage plant is designed for carrying out experiments on a small scale. The outer dimensions of the plant are $14\frac{3}{4}$ ft. \times $7\frac{1}{2}$ ft. \times $7\frac{3}{4}$ ft. and has a storage capacity of one and a half tons. It consists of three small chambers designed to maintain three different temperatures (Plate XXX, fig. 1). The chambers are at present being cooled by cool air circulated by a fan over cooling coils. Each chamber is divided into four compartments and each compartment is fitted with three removable shelves. Individual chambers are served by an independent compressor-motor connected to a thermostat switch. There is an 'air-lock' $4\frac{1}{2}$ ft. \times $2\frac{1}{2}$ ft. \times 7 ft. for each chamber.

Di-chloro-di-fluoro-methane commercially known as Freon or F-12 is used as refrigerant. It is non-inflammable and non-poisonous.

The plant was installed at the end of October, 1937. Since then several additions and alterations have been effected to get a closer and more uniform control of temperature. Most of the changes have been in the direction of electric installations done to get different speeds of circulating air. The diagrams of the electric installation in the beginning and at present are given in Fig. 1. In the present arrangements it is possible to control the air speeds, both when the compressor is working and when it is at rest. These controls are adjustable according to the requirements as to whether higher speed is required when the compressor is working or when it is at rest. This arrangement helps to minimize the fluctuations at the top and bottom of the chambers.

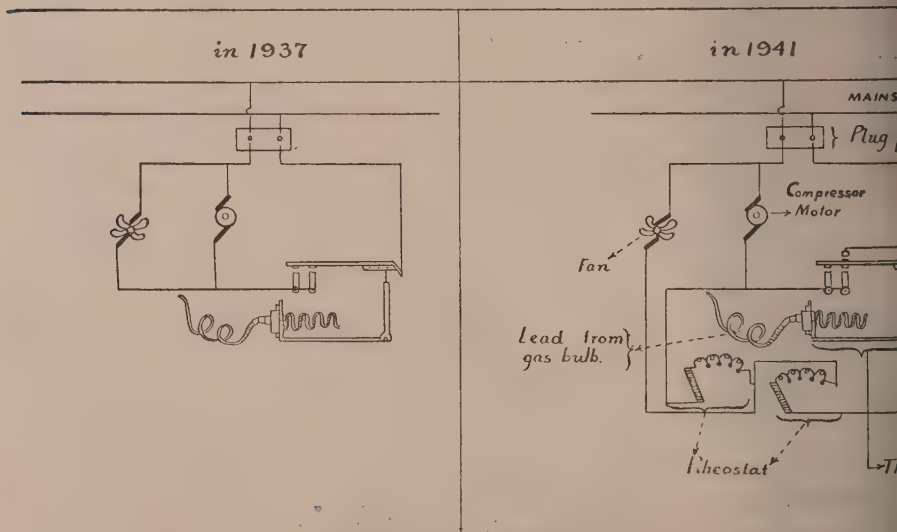


FIG. 1. Plan of electric installation in the cold storage plant



FIG. 1. Cold storage plant at Lyallpur

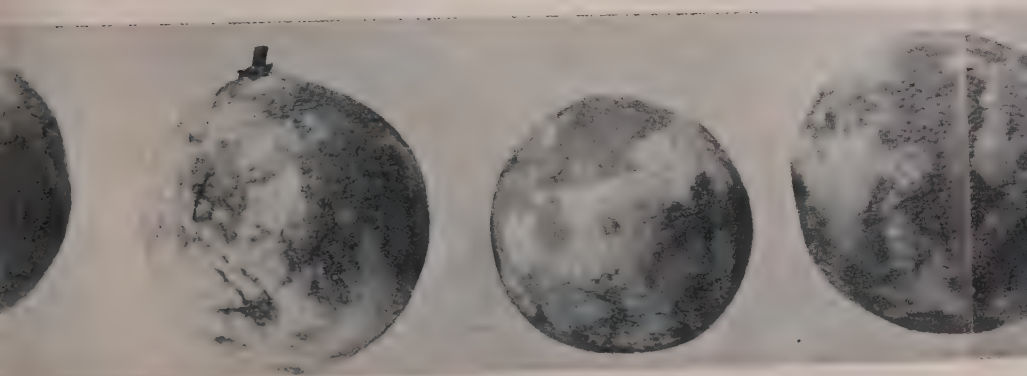


FIG. 2. Chill frost injury of fruit.

METHOD

The experiment was arranged to study the effect of size of fruit and temperatures upon the storage life of different varieties of Malta and Sangtra oranges.

The fruit was graded into two sizes, viz. large and small. The diameter of large fruit varied from 7.2 cm. to 8.9 cm. and small fruit from 6.0 cm. to 7.1 cm. Half the number of fruits of each size was wrapped with butter-paper and the other half kept unwrapped. The fruit after wrapping was subjected to different ranges of storage temperature. The ranges of temperature in different chambers during the first trial were 24°-32° F., 36°-39° F. and 40°-43° F. But the fluctuations were reduced to a closer limit due to the provision of more piping and improvement in the air circulation, different temperatures ranged as 29°-32° F., 36°-39° F. and 40°-43° F.

The number of fruits used under each treatment (viz. three temperatures, two sizes and two wrappings) was 72 and the fruits were arranged in two rows in the trays. The trays were fitted with wooden splints at the base.

Losses in weight of fruit

For the purpose of determining the loss in weight during storage six additional fruits under each treatment were numbered and weighed individually at fortnightly intervals.

Other physico-chemical analyses

Observations in case of all the varieties stored were made on the general condition of the fruit after every two weeks. Percentage weights of peel and pulp juice were recorded as well as acid and sugar (total soluble solid) content of the juice determined [Trout *et al.* 1938] at four-week intervals. Four samples were taken at random from each sub-lot at each occasion and the juice was extracted with the help of an electric driven 'Rose's cone' and then filtered through a muslin piece with hand press.

Storage life. The fruit was considered properly stored so long as the wastage did not exceed 10 per cent.

Keeping quality of fruits after removal from cold store. Occasionally four samples stored at each temperature were taken out of the cold store and placed at room temperature to study their keeping quality after removing from cold storage.

Study of rot organisms. A study of rot organisms was made and identification was carried out.

RESULTS

The results obtained in case of Malta and Sangtra oranges and even of other varieties of Malta orange are in general the same excepting their storage life. Consequently the data mainly of one variety, viz. Common Malta (of other varieties wherever necessary) are presented to economize space. Data relating to storage life of different varieties of Malta and Sangtra are given in each case. The temperature range of 29°-32° F. being found to be unsuitable for the storage of oranges due to the development of rot spots, the data at this temperature range were not collected.

EFFECT OF STORAGE TEMPERATURE, SIZE OF FRUIT, WRAPPING AND PE
OF STORAGE

GENERAL CONDITION AND WASTAGE OF THE FRUIT

At 29°-32°F. the fruit developed chill spots (Plate XXX, fig. 2) couple of weeks in storage. The spotting was invariably accompanied by deterioration in taste to a varying degree which ranged from flat-wat to bitter and abnoxious. Even fruit, without chill spots at this temperature deteriorated in taste. This deterioration in taste was more marked when fruit was placed at room temperature (90°-100°F.) for a few hours. Chill trouble was the least in case of Valencia Late and the most in Blood Red.

36°-39°F. proved to be the best range of temperature for the storage of Malta and Sangtra oranges. Malta Common at this temperature kept in excellent condition for four months (Table I), Blood Red for three months, Valencia Late for four and a half months, Seville for three months and Musambi 2½ months (Fig. 2). Sangtra from Lyallpur and Pathankote kept in excellent condition for seven weeks in 1938 but the storage life was reduced to four weeks respectively in 1939 as the fruit was subjected to greater abuse of handling and was not carefully picked and packed by the grower.

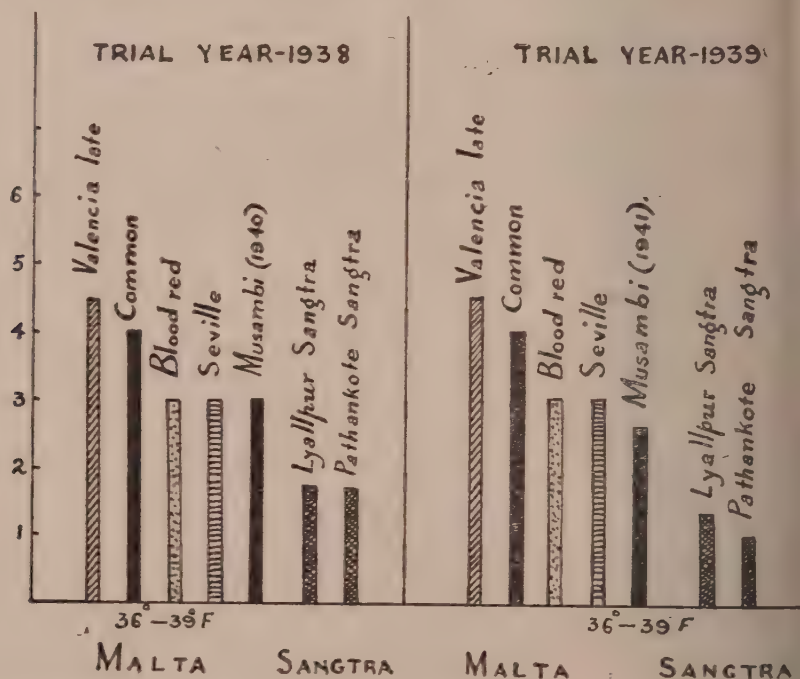
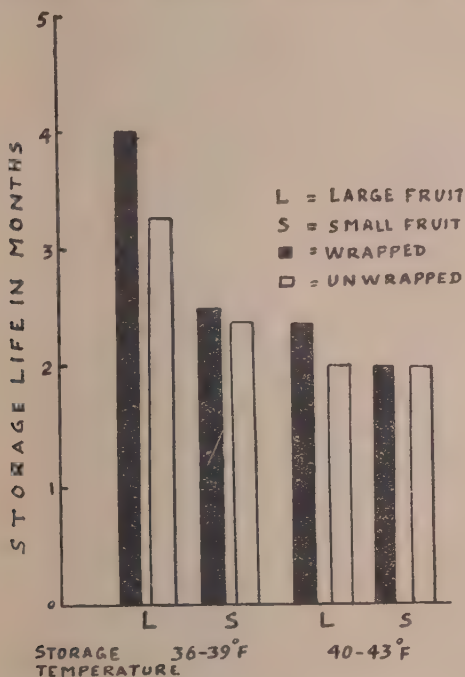


FIG. 2. Showing the maximum cold storage life of different varieties of Malta and Sangtra under optimum conditions

the storage life of the Malta orange of different varieties was reduced at 40°-43°F. both due to fungal attack and shrivelling.

The size of the fruit had considerable effect on the storage life of the fruit, large fruit kept in good condition for a longer period as compared to small fruit (Fig. 3).

All fruits presented shrivelled appearance earlier than the large fruits. The storage life was thus considerably shortened in case of small fruits (Table I). However, wrapping the fruit with butter paper proved to be beneficial in preserving the colour, brilliancy and freshness of the skin of all the varieties tried.



Showing the storage life of Malta common fruit under different treatments

WEIGHT OF FRUITS (Tables II, III, IV & V)

The per cent total loss in weight of fruit (calculated on original fresh weight of fruit) was determined from time to time. It was observed that the weight increased with the advance in storage period. The loss in weight was greatest at 40°-43°F. and the least at 29°-32°F. and unwrapped fruit lost more weight than wrapped one. Similarly, small fruit lost comparatively more weight than large one. At the end of four months storage the total loss in weight of fruit under optimum conditions (large wrapped fruit, stored at 29°-32°F.) varied from 18 per cent (in Valencia Late) to 25 per cent (in Malta). Blood Red lost 25 per cent of its weight after three months (Table II).

PHYSICO-CHEMICAL CHANGES

Physico-chemical analyses of fruit were carried out for per cent of peel, per cent weight of juice, acidity and total soluble solids.

TABLE I

Showing the effect of temperature, size of fruit, wrapping, and period of on the per cent rate of wastage of fruit, of Malta Common orange

(Calculated on the actual number of fruits in storage)

No. of days in storage	Large				Small		
	Wrapped		Unwrapped		Wrapped		Unwrap
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.
0	0	0	0	0	0	0	0
30	0	2.78	1.39	4.16	0	1.39	0
45	0	0	1.52	0	1.49	0	0
60	0	3.07	1.54	4.69	1.52	3.03	1.49
75	0	10.30	0	18.70	6.66	35.60	9.84
90	1.54	9.61	5.08	17.40	17.87	13.16	23.64
105	3.57	54.78	33.30	72.72	58.55	...	67.50
120	7.40	...	17.65	...	75.0	...	66.60

Malta Common orange fruit
(Calculated on original fresh weight)

No. of days in storage	Large						Small					
	Wrapped			Unwrapped			Wrapped			Unwrapped		
	29°-32°F.	40°-43°F.		29°-32°F.	36°-39°F.		29°-32°F.	36°-39°F.		29°-32°F.	36°-39°F.	
		36°-39°F.	40°-43°F.		36°-39°F.	40°-43°F.		36°-39°F.	40°-43°F.		36°-39°F.	40°-43°F.
0	0	0	0	0	0	0	0	0	0	0	0	0
30	2.6	4.15	5.15	3.22	5.33	6.10	3.04	4.84	5.82	5.16	6.45	7.00
45	...	8.63	9.04	...	9.88	10.13	...	9.97	8.71	...	10.72	12.03
60	...	12.82	14.27	...	13.35	14.20	...	14.50	15.20	...	15.23	16.71
75	...	19.80	22.40	...	20.18	23.40	...	21.43	22.35	...	22.10	24.20
88	...	21.72	27.50	...	23.32	28.10	...	23.50	27.38	...	25.0	29.10
104	...	24.05	32.00	...	26.57	33.50	...	26.02	31.50	...	28.66	34.82
120	...	27.10	35.87	...	29.83	38.37	...	31.48	35.94	...	33.30	41.70

TABLE III

Showing the mean per cent total loss in weight of large sized fruit of different varieties of Malta orange under optimum conditions at the end of storage

Varieties	Storage life	Year	
		1938	1939
		36°-39°F. (per cent)	36°-39°F. (per cent)
Common	4 months	25.5	27.5
Valencia Late	4½ months	19.1	17.5
Blood Red	3 months	25.5	21.5
Seville	3 months	22.6	21.5
Musambi*	2½ months	23.5	19.5

* Musambi was tried during 1940 and 1941

TABLE IV

Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent loss in weight of fruit of Blood Red orange

(Based on six numbered fruits)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
15	4.8	4.16	4.5	6.97	5.97	6.66	5.85	6.97
30	11.77	12.50	9.10	11.65	11.65	13.58	16.67	16.67
45	13.70	16.66	15.90	16.98	16.28	21.63	20.22	20.22
60	17.64	20.92	18.18	21.93	20.94	24.02	24.22	24.22
75	21.50	25.00	25.00	27.91	23.26	29.43	27.78	27.78
90	25.48	29.17	31.80	39.53	27.91	35.43	36.10	36.10
105	28.50	33.17	35.80	44.34	32.81	39.56	38.30	38.30

TABLE V

Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent loss in weight of fruit of Valencia Late orange

(Based on six numbered fruits)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
1	0.5	2.20	0.9	1.20	2.50	2.50	1.20	2.70
2	2.10	2.40	2.30	2.60	3.12	4.00	2.80	4.97
3	4.25	6.00	4.40	6.20	5.12	10.00	5.10	8.10
4	6.08	8.00	6.90	8.90	7.09	15.50	8.90	17.50
5	8.51	11.11	11.11	13.40	12.80	18.00	10.30	21.60
6	11.70	15.50	15.50	17.38	13.02	20.80	14.80	22.80
7	14.90	17.78	20.00	21.74	15.38	21.90	18.00	24.30
8	16.50	19.70	21.00	23.20	18.00	23.00	21.30	26.20

(i) *Per cent weight of peel* (Tables VI-VIII, Fig. 4). The per cent weight of peel was calculated on original fresh weight of fruit [Martin, 1937]. It decreased with the advance in the period and the decrease was more at 43°F. than at 36°-39°F. The per cent weight of peel was higher in large fruits than in small ones to start with, and remained so throughout the period of storage. The weight of peel decreased more in unwrapped fruits than in wrapped ones.

(ii) *Per cent weight of available juice* (Tables IX-XI, Fig. 4). Figures for available juice in the fruit were calculated on the original fresh weight of the fruit [Martin, 1937]. The per cent weight of juice decreased with advance in the storage period. The fruits stored at 36°-39°F. had higher juice content than those at 40°-43°F. after a storage of four months. All fruits had higher juice content than large ones, at the beginning of storage, but lost more weight of juice than large ones at the optimum storage temperature, i.e. 36°-39°F. Wrapped fruits had higher juice than unwrapped fruits.

(iii) *Acid and total soluble solid contents of the juice* (Tables XII and XIII). The acid and total soluble solids decreased during the period of storage when calculated on the original fresh weight. Other treatments,

viz. size, wrapping and temperature did not exhibit any marked differences the total soluble solid content though acid contents at 40°-43°F. were lower than that at 36°-39°F. after four months storage.

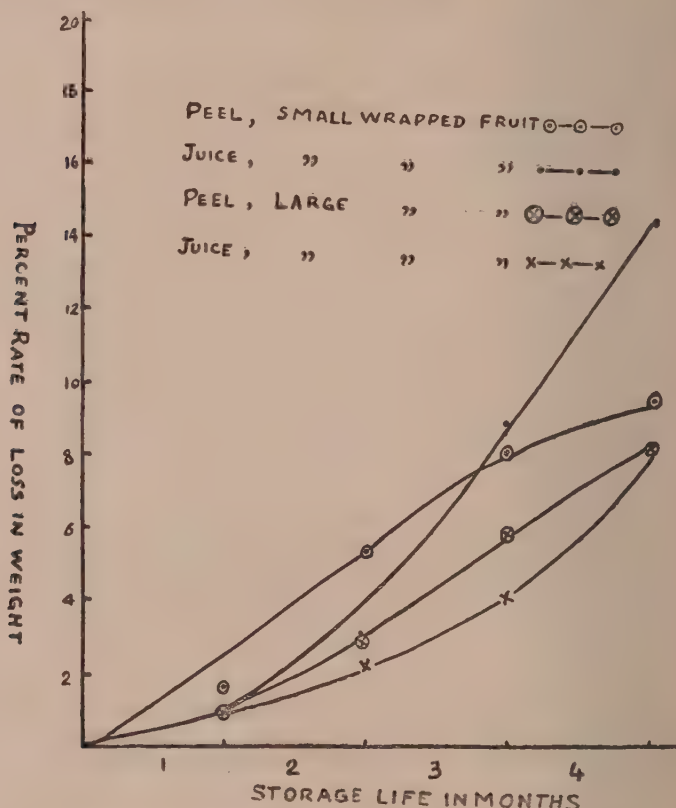


FIG. 4. Showing comparative loss in weight of peel and juice of Malta orange during storage under optimum conditions

FUNGAL PATHOGENS

Malta oranges

Chill spotting and deterioration in taste were mainly responsible for spoilage at low temperature, viz. 29°-32°F. But at higher temperatures (36°-39° and 40°-43°F.) the wastage was mostly due to the fungal attack, viz. *Penicillium digitatum* and *P. italicum*. The first symptom was the softening of the tissue followed by a visible spread of the fungus. *Alternaria* sp. was also isolated from a few fruits. The fungus in this case was observed on the internal segments of the pulp near the stem end. In 1939 storage trials, *Colletotrichum gleosporioides* penzig was also observed to cause the stem end rot.

Common orange fruit
(Calculated on original fresh weight)

No. of days in storage	Large						Small			
	Wrapped			Unwrapped			Wrapped		Unwrapped	
	36°-39°F.		40°-43°F.	29°-32°F.		36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	40°-43°F.
	29°-32°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	29°-32°F.	36°-39°F.	40°-43°F.
0	34.48	34.48	34.48	34.48	34.48	28.15	28.15	28.15	28.15	28.15
30	33.57	33.48	32.32	33.01	30.30	27.50	26.30	28.40	24.0	24.38
60	...	31.50	26.20	...	28.60	...	22.57	...	20.04	19.34
90	...	28.56	21.01	...	25.18	...	19.83	...	17.85	14.23
120	...	25.95	17.55	...	21.55	...	18.33	...	15.82	13.56

TABLE VII

Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of peel of Blood Red orange fruit
(Calculated on original fresh weight)

No. of days in storage	Large						Small			
	Wrapped			Unwrapped			Wrapped		Unwrapped	
	36°-39°F.		40°-43°F.	36°-39°F.		40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
	36°-39°F.	40°-43°F.	40°-43°F.	36°-39°F.	40°-43°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0	34.03	34.03	34.03	34.03	34.03	31.5	31.5	31.5	31.5	31.5
45	28.5	27.85	27.5	27.5	25.1	26.02	23.80	22.97	20.30	22.97
75	24.20	24.02	22.1	22.1	20.20	21.50	18.90	18.90	19.30	18.90
105	21.42	19.08	17.36	17.36	14.35	17.60	14.97	15.83	12.5	12.5

TABLE VIII
Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of peel of Valencia Late orange
 (Calculated on original fresh weight)

No. of days in storage	Large						Small					
	Wrapped			Unwrapped			Wrapped			Unwrapped		
	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.
0	34.52	34.52	34.52	34.52	34.52	34.52	29.78	29.78	29.78	29.78	29.78	29.78
30	...	34.00	34.68	...	32.58	32.20	...	27.78	28.20	...	27.80	26.90
60	...	30.02	29.64	...	28.77	29.28	...	27.40	24.24	...	26.78	23.08
90	...	28.59	27.82	...	25.34	26.98	...	25.10	21.22	...	24.88	19.19
120	...	26.20	22.48	...	24.12	17.28	...	21.45	19.20	...	19.90	16.64

TABLE IX.
Showing the effect of temperature, size of fruit, wrapping and time of storage on the per cent weight of available juice
of Malta Common orange fruit

(Calculated on original fresh weight)

No. of days in storage	Large						Small					
	Wrapped			Unwrapped			Wrapped			Unwrapped		
	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.
	44.13	44.13	44.13	44.13	44.13	44.13	52.07	52.07	52.07	52.07	52.07	52.07
0	44.13	44.13	44.13	44.13	44.13	44.13	52.07	52.07	52.07	52.07	52.07	52.07
30	43.55	43.27	43.42	43.25	42.80	42.60	50.85	51.20	47.57	49.07	49.34	46.14
60	...	42.00	40.70	...	39.00	38.90	...	43.58	46.27	...	46.10	44.77
90	...	39.95	37.06	...	37.70	35.40	...	43.48	41.05	...	41.05	39.30
120	...	36.04	31.04	...	33.16	28.73	...	37.20	33.85	...	35.33	31.40

TABLE X

Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of available juice of Blood Red orange

(Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0 . . .	48.91	48.91	48.91	48.91	51.4	51.4	51.4	51.4
45 . . .	43.15	41.55	41.9	41.2	43.78	44.2	44.9	44.9
75 . . .	40.66	37.78	38.7	36.64	41.2	40.45	40.5	40.5
105 . . .	36.13	34.57	33.43	29.84	38.4	32.08	34.25	34.25

TABLE XI

Showing the effect of temperature, size of fruit, wrapping and period of storage on the per cent weight of available juice of Valencia Late orange

(Calculated on original fresh weight)

No. of days in storage	Large				Small			
	Wrapped		Unwrapped		Wrapped		Unwrapped	
	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.	36°-39°F.	40°-43°F.
0 . . .	48.7	48.7	48.7	48.7	52.77	52.77	52.77	52.77
30 . . .	49.0	46.4	46.5	46.36	53.1	52.0	52.5	52.5
60 . . .	48.4	47.20	48.2	44.6	50.14	46.4	48.7	48.7
90 . . .	45.09	44.8	44.87	44.6	47.02	45.10	46.5	46.5
120 . . .	44.73	42.5	42.2	41.7	47.0	45.4	45.2	45.2

TABLE XII

Showing the effect of temperature, size of fruit, wrapping and time of storage on the acid contents of Malta Common orange fruit

(Calculated on original fresh weight)

(Acidity given in grammes of citric acid per 100 c.c. juice)

No. of days in storage	Large						Small		
	Wrapped			Unwrapped			Wrapped		Unwrapped
	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.	29°-32°F.	36°-39°F.	40°-43°F.
0	0.67	0.67	0.67	0.67	0.67	0.67	0.68	0.68	0.68
30	0.60	0.69	0.55	0.65	0.64	0.49	0.60	0.53	0.63
60	...	0.56	0.54	...	0.53	0.55	0.55	0.55	0.53
90	...	0.54	0.51	...	0.54	0.45	0.45	0.46	0.43
120	...	0.44	0.39	...	0.56	0.44	0.41	0.43	0.41

TABLE XIII

Showing the effect of temperature, size of fruit, wrapping and time of storage on the total soluble solids of juice of
Maltia Common orange fruit
 (Calculated on original fresh weight)

No. of days in storage	Large						Small					
	Wrapped			Unwrapped			Wrapped			Unwrapped		
	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)	29°-32°F. (per cent)	36°-39°F. (per cent)	40°-43°F. (per cent)
0	9.30	9.30	9.30	9.30	9.30	9.30	9.80	9.80	9.80	9.80	9.80	9.80
30	8.57	9.30	9.58	8.83	8.08	9.58	9.31	9.70	9.79	9.84	8.60	10.88
60	...	8.11	8.32	...	8.14	8.95	...	8.72	9.16	...	9.15	10.26
90	...	8.45	7.97	...	8.43	8.62	...	8.29	7.99	...	7.80	8.02
120	...	7.29	8.08	...	8.11	8.01	...	8.75	8.20	...	7.85	8.68

ORANGES

Sangtra, in addition to blue and green moulds, another fungus *S. niger* was also found on decaying fruits.

KEEPING QUALITY OF THE FRUIT AFTER REMOVAL FROM COLD STORE

Malta oranges were occasionally removed from cold store and placed at room temperature (86°-110°F.) to see the keeping quality of the fruit after removal from the cold store. It was observed that the keeping quality of the fruit at room temperature was dependent upon the period of storage and the temperature of the room at which the fruit was stored. The longer the period, the shorter was its keeping quality. Again, the room temperature at which the fruit was placed after removal from cold store, the shorter it kept in marketable condition. The observations are given in Table XIV.

TABLE XIV

Temperature and period of storage on the keeping quality of Malta orange fruit after removal from cold store

Temperature at which fruit was placed	Period of storage after which the fruit was removed from cold store	Storage temperature of fruit	Keeping quality of fruit after removal from cold store
	1 month	40°-43°F. 36°-39°F. 29°-32°F.	12 days 12 days 2 days in case of unfrozen fruit. Fruit bitter
F.	2 months . . .	40°-43°F. 36°-39°F. 29°-32°F.	10 days 7 days Fruit bitter in taste
F.	3 months . . .	40°-43°F. 36°-39°F.	5 days 5 days
F.	4 months . . .	36°-39°F.	3-4 days
F.	4½ months . . .	36°-39°F.	2-3 days

DISCUSSION OF THE RESULTS

In the present investigations the best range of temperature for the storage of Sangtra and Sangtra oranges was found to be 36°-39°F. (air temperature of storage chamber). As already mentioned under 'review of literature', the observations of various workers as to the optimum temperatures for storage of oranges vary from 32°-50°F., depending upon various considerations. While temperatures above and below the recommended temperatures are bound to result in different kinds and varieties of citrus fruits, difference in soil, cultural operations, age of trees, stage at which fruit is picked, care

in handling and storage conditions, etc. yet, 36°-39°F. temperature taken as quite safe for the storage of Malta and Sangtra oranges under Punjab conditions.

At the lower temperature range (29°-32°F.) the fruit showed signs of collapse—a malady known as 'chill spot' injury of the fruit. The fruit thus affected lost its market value. In addition to this, the fruit at this temperature tasted bitter, probably due to the liberation of the bitter principle oranges the 'Limonin' [Higby, 1941].

At higher temperatures (40°-43°F.) the storage life of the fruit was considerably shortened due to the appearance of fungal diseases and shrivelling of the fruit.

The size of the fruit is an important factor in determining the storage life of the fruit. The present investigations reveal that large sized fruit kept longer and in better condition and lost less weight than small sized fruit. This is in conformity with the results obtained by other workers referred to under 'review of literature'.

Wrapping the fruit with butter-paper proved beneficial in reducing the loss in weight of fruit and also preserved the brilliancy and freshness of the fruit. No 'suffocation' of the fruit resulting in deterioration of the fruit as mentioned by Williams [1938] was observed in wrapped fruit as the covering of the butter-paper was not so air-tight as to completely obstruct the exchange of gases. Another advantage of wrapping the fruit was that the fruit getting diseased remained isolated from healthy ones. Wrapping the fruit has also been recommended by other research workers, referred to under 'review of literature'.

The storage life of different varieties of Malta orange was found to vary considerably. Malta Common kept well for four months, Valencia I for three months, Blood Red three months, Seville three months and Musambi 2½ months under optimum conditions of storage (36°-39°F.). The study of literature on the subject reveals that storage life of even the same variety, varies under different conditions and thus no absolute limit can be laid down as to the actual storage life of the fruit. The present investigations, therefore, indicate the limits around which the storage life of a particular variety would vary, as the extent of storage life is influenced by so many factors, as mentioned.

The storage life of the Punjab Sangtra orange was considerably longer (4-7 weeks) than Malta orange which is probably due to the 'puffy' nature of the fruit, which makes it liable to damage very easily in transit or in handling. The storage life of King orange has been reported to be 50 days at 45°F. Nagpur orange 90 days at 40°F. but these varieties are not cultivated in Punjab and are far more tight skinned than the Punjab Sangtra orange.

Physico-chemical analyses of the fruit showed that the loss in weight was both from peel and juice during the storage period. In the beginning of the storage life, peel lost weight to a greater extent than the juice, while at the end of the storage life reverse was the case. This probably is due to the fact that the peel being more turgid in the beginning, readily lost its moisture before the juice could be affected. Small fruits lost more weight than large ones. This has been observed by other workers, also, referred to under 'review of literature'.

is due to the fact that in small fruits, the surface exposed per unit of volume is more than in large fruits.

The acid and total soluble solid contents of the juice were not affected in proportion to the decline in taste and consequently only acid and total soluble solid contents cannot be true index of quality. The acid and total soluble solids showed a decrease during the period of storage when calculated on original fresh weight and this is also observed by other workers cited under 'review of literature'.

Fruit removed from cold storage and placed at room temperature showed that the keeping quality of the fruit at room temperature decreased with the advance in the period of storage. This is probably due, partly to the rise in temperature of the room during summer and partly to low resistance of the fruit to withstand high temperatures after prolonged storage at low temperature.

SUMMARY

The investigations reported in the paper were carried out during 1938 and 1939 at Lyallpur under the Research Scheme on the Cold Storage of Fruits in the Punjab, financed jointly by the Imperial Council of Agricultural Research and the Punjab Government. Results of investigations on Malta (*Citrus sinensis*) and Sangtra (*C. nobilis*) obtained during the above period may be summed up as under :—

1. Five varieties of Malta, viz. Valencia Late, Common, Blood Red, Seville and Musambi and Sangtra from two localities namely Lyallpur and Pathankot were stored at three storage temperatures, viz. 29°-32°F. 36°-39°F. and 40°-43°F. Large and small fruits of each variety were used. Half of the fruit was wrapped with butter-paper and the other half stored as such.
2. Physico-chemical analyses were carried out at regular intervals.
3. The best temperature range for the storage of citrus fruits (Malta and Sangtra) was found to be 36°-39°F.
4. The storage life of Malta (*C. sinensis*) varied with varieties, (a) Valencia Late kept in good condition for 4½ months, (b) Common for four months, (c) Blood Red for three months, (d) Seville for three months and (e) Musambi for 2½ months.
5. Loose skinned Sangtra from Lyallpur and Pathankot kept in good condition for five and four weeks respectively.
6. Large fruit kept longer and in better condition than small fruit.
7. Wrapped fruit presented better appearance in regard to its colour and freshness and had higher juice content and lower wastage than unwrapped fruit.

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CAJANUS OBCORDIFOLIA SINGH

A NEW SPECIES OF CAJANUS

BY

D. N. SINGH

Lecturer in Botany

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(Received for publication on 19 May 1941)

(With six text-figures)

CAJANUS is a monotypic genus, represented in India by the only species *Cajanus cajan* Millsp. (*Cajanus indicus* Spreng.—Hindi—*arhar*). The two sub-species are *C. indicus flavus* and *C. indicus bicolor*, the differentiation being based on the colour of the flower and the habit of the plant. This plant is extensively cultivated in the Gorakhpur district of the United Provinces. In December 1939 the senior author noted in one of the *arhar* fields a plant which was distinctly different from the others, and yet, it appeared to resemble *cajanus*. At that time all branches except one were flowerless. The plant was allowed to seed, which was later on collected and brought to Cawnpore and sown in the Botanical Garden of the Agricultural College. For comparison a plot of normal type of *arhar* was also sown.

All the plants of this new type, which were about 50 in number, repeated without exception the characters of the mother plant. The chief characters of the new type of *arhar* and that of the normal *C. cajan* Millsp. are summarized in Table I given below :—

TABLE I

Character	<i>Cajanus cajan</i> , Millsp.	<i>Cajanus</i> (New type)
Leaflets	Trifoliate	Trifoliate
Leaf shape	Lanceolate	Obcordate
Leaf apex	Acute to slightly acuminate	Retuse and mucronate
Stemular hair	Numerous and prominent	Comparatively very few
Lower petals	Yellow	Yellow; lighter in colour
Seal	United at the top	Quite free in open flower, filiform and usually appendaged
Flower	Lobes one sided and asymmetrical with a pronounced peg-like out-growth at the base; veins more prominent.	Lobes present on both the sides and hence symmetrical in shape, with a less pronounced peg-like out-growth at the base; veins comparatively inconspicuous

It is evident from the comparative description that the new type differs from the normal type in having small and obcordate leaflets with retuse mucronate apices, while the normal type has oblong lanceolate leaflets (Fig. 1 and 2). On the basis of 100 observations made regarding the central leaflet the following interesting data have been obtained and are shown in Table II and Fig. 3.

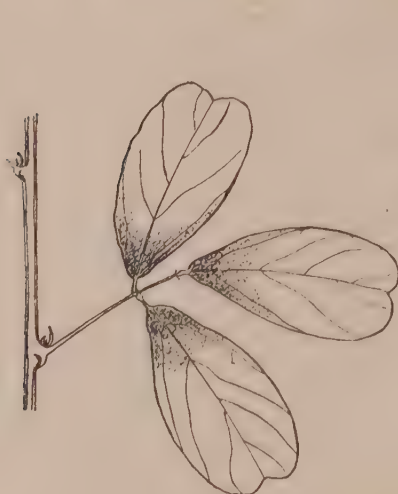


FIG. 1. Showing *Cajanus* (New type)

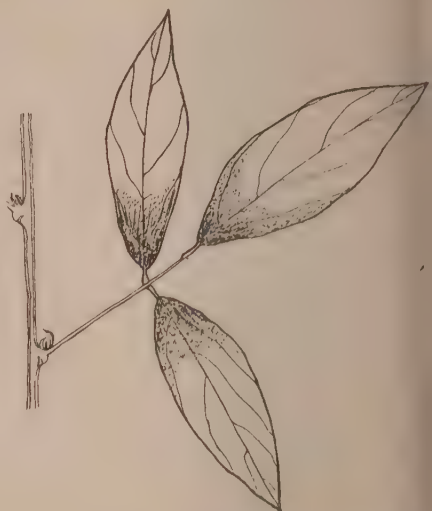


FIG. 2. Showing *Cajanus cajan* (L. Millsp.) (*Cajanus indicus* Spreng.)

TABLE II

Name	Variation in the length of central leaflets in mm.	Mean length of central leaflet in mm.	Variation in the width of central leaflets in mm.	Length Width ratio
<i>C. cajan</i>	89-110	97.2	35-43	2.3-2.8
<i>C. obcordifolia</i>	63-76	68.6	31-40	1.5-2.1

The structure of the flowers is also very different; the keel in the new type of *arhar* is free and represented by two filiform lobes usually appendaged while in the normal type the lobes are broad and united at the top (Figs. 4, 5 & 6). The alae or wings in the normal type are one-sided and asymmetrical having a very pronounced peg-like outgrowth at the base, whereas, these in the new type are symmetrical having lobes on both the sides with a less pronounced peg-like outgrowth.

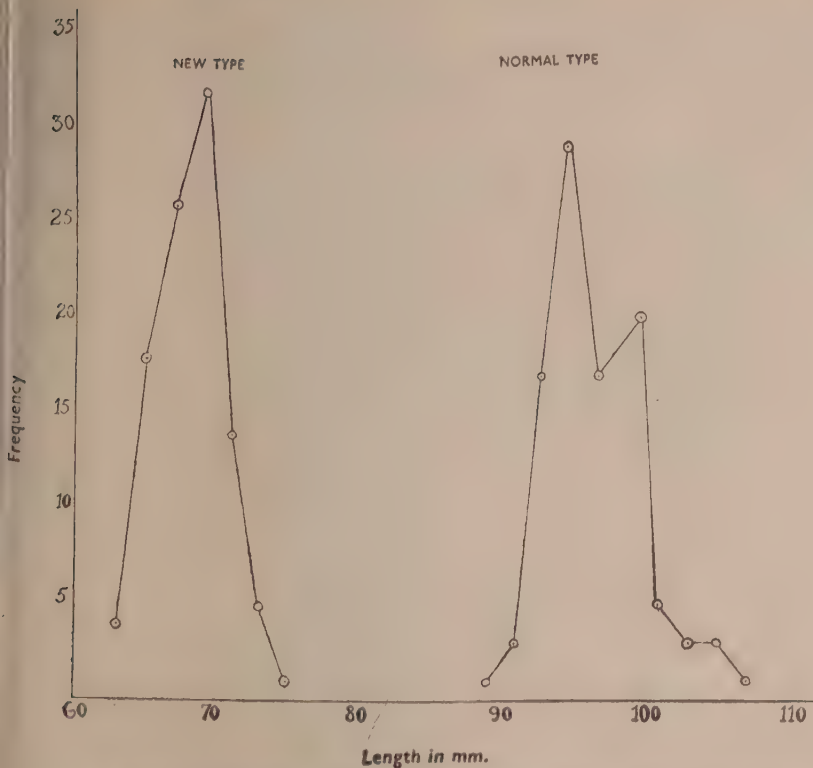
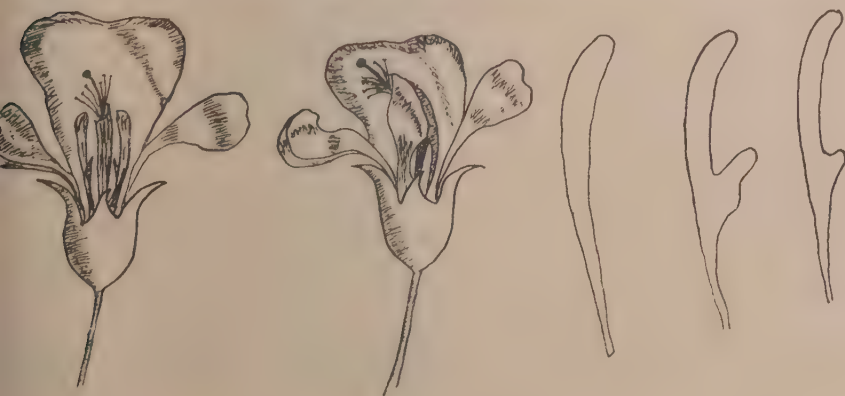


FIG. 3. Showing frequency distribution of central leaflet length in normal and new type



Keel petals free

Keel petals united

Keel petals new type

FIGS. 4, 5 and 6. Showing the structure of flowers of *Cajanus* (New type) and *Cajanus cajan* (L.) Millsp. (*Cajanus indicus* Spreng.)

According to analytical key given by Bailey [1924] the plant should long to the genus *Cajanus*. The description of *Cajanus indicus* Spreng., by Hooker [1875] states that the leaflets are oblong—lanceolate; Bailey states that they are lanceolate to narrow—clleptical. Hooker's classification is silent about the keel, while Bailey describes it as 'obtuse and incurved'. Keel is a very distinctive character of Leguminosæ—Papilionaceæ. The deviation from the normal keel been noted in *Cajanus cajan* it would have been emphasized. But so far no reference in the literature on the subject is to this fact.

During the course of above investigation, a study of the chromosome number of this new type was also made. Aceto-carmene smears of pollen mother cells were prepared for determining the gametic chromosome number. Buds of about 3 mm. in length were found to be the best for the study of meiotic chromosomes in the pollen mother cells at the first metaphase. Haploid number of chromosomes was found to be eleven.

The study of the somatic chromosomes from the root-tips of the germinating seeds was also made. The root tips were fixed in Flemming's chromosmium-acetic combination (weaker solution) and after the usual process of embedding and cutting, the sections were stained by Newton's Gentian Violet Iodine method and mounted in Canada balsam. The number of somatic chromosomes was found to be $2n=22$.

This new type of *Cajanus* may be the result of gene-mutation. Further investigations on other features of genetical and cytological interest are in progress. In the meanwhile owing to certain marked differences in vegetative and floral characters in this new type of *Cajanus*, it may be considered as a new species and is tentatively named as *Cajanus obcordifolia* Singh after its distinguishing character, viz. the obcordate shape of the leaflet.

Botanical description of *Cajanus obcordifolia* Singh. in English

Plant :	. . .	Erect and shrubby
Root :	. . .	Tap ; fibrous and branched
Stem :	. . .	Erect ; woody ; cylindrical and ribbed with many shallow sulcate and grey silky branches
Stipule :	. . .	Minute ; lanceolate ; fugacious
Leaf :	. . .	Alternate ; compound ; imparipinnate ; with channelled petiole ; trifoliate
Leaflet :	. . .	Stalked, minutely stipellate, reticulate, obcordate with mucronate apex ; margin entire ; glabrous ; deep green above and whitish and pubescent below
Inflorescence :	. . .	Indefinite ; corymbose raceme ; often forming a terminal umbel
Flower :	. . .	Irregular ; hermaphrodite ; pedicellate ; pedicels prostrate, hairy and two to three times the calyx. The floral bud has a crumpled tip and not unoften there is an opening at the extreme tip
Calyx :	. . .	Gamosepalous ; campanulate ; persistent ; glandular ; persistent ; teeth short ; inferior

- Irregular; polypetalous; papilionaceous; more than twice as long as the calyx; perigynous; standard yellow; alæ or wings with lobes on both the sides and more or less symmetrical in shape; peg-like outgrowth (auricle) at the base less pronounced; clawed; veins inconspicuous. Keel petals light in colour; Quite free in open flower; filiform and usually appendaged
- Andræcium: Ten in two bundles (9) + 1 (diadelphous); not enclosed in keel in open flower; anthers uniform. These dehisce in the bud even before the flower has reached its maximum size. This early dehiscence in this type may be due to the small opening at the tip resulting in greater loss of moisture from the anther walls
- Gynæcium: Monocarpellary; subsessile; few ovuled; superior; with a long filiform and upcurved style; stigma capitate; ovary wall pubescent; pod with conspicuous black splashes (markings); beaked and constricted between the seeds; hairy; seed exalbuminous; compressed; smooth and of light colour

Botanical description of *Cajanus obcordifolia* Singh. in Latin

Cajanus obcordifolia Singh. sp. nov.

Frutex erectus. Radix principalis fibrosa et ramosa. Truncus erectus, cylindricus et multis tenuibus, sulcatis et cinereo-sericeis ramis costatus foliis minuti, lanceolati, decidui. Folia alterna, composita, imparipinnata, sulcato petiolo prædita, trifoliata. Foliola petiolata, minute stipellata, ovate, apice retuso et mucronato; margine integra; superiore facie glabra, viridi, inferiore vero albescente et pubescente. Inflorescentia indurata, corymbosa racemosa; sæpe terminaliter paniculata. Flores irregulares, papilionaceae, pedunculati; pediculi profuse pilosi, et bis vel ter calyce longiores. Legumen gemma est corrugata apice et non raro in extremo apice foramine prædita. Calyx gamosepalus, campanulatus, persistens, glandulis præditus, lobis scissis, inferior; calycis dentes breves. Corolla irregularis, polypetala, papilionacea, plus bis longior calyce, perigyna; vexillum flavum; alæ lobatae, longioribus rostris latere et plus minus symmetricæ forma; auricula clavo similis in apice minus conspicua; petala ad basim tenuescencia; venæ inconspicuæ. Petala colore claro, omnino libera in aperto flore, filiformia et sæpe appendiculata. Andræcium; stamina diadelphe (9 + 1); non inclusa in aperto flore; antheræ uniformes, dehiscentes in gemma etiam in aperto quam flos maximam magnitudinem attigerit. Præmaturæ huius dehiscence causa forte sit parvum illud foramen in apice gemmæ, quod efficit ut antheræ parietes majorem humiditatis quantitatem amittant. Gynæcium monocarpum, subsessile, superius, paucis ovulis et stylo longo filiformi et in apice curvato præditum; legumen conspicuis nigris maculis ornatum, rostrato et constrictum inter semina; pilosum. Semen exalbuminatum, compressum, planum vel læve et colore clarum.

ACKNOWLEDGEMENTS

Our thanks are due to Dr T. S. Sabnis, I.A.S., Economic Botanist to Government, United Provinces, and Principal, Agricultural College, Cawnpore,

and Mr P. R. Mehta, Assistant Professor of Botany, for their helpful
tions, and to Mr. T. R. Mehta for his unfailing interest and kind criticism
the course of the above investigation. We feel greatly indebted to I
Santapan of the St. Xavier's College, Bombay, for the Latin description
new species.

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RESEARCH NOTE

CHROMATIN BRIDGES IN COTTON*

BY

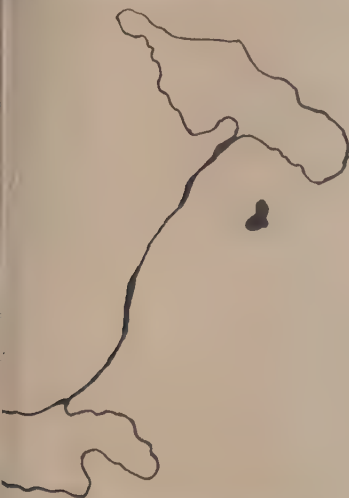
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Agricultural Research Station, Surat

(Received for publication on 7 January 1942)

(With four text-figures)

CHROMATIN bridges were noticed at anaphase I, metaphase II and anaphase II of meiosis (Figs. 1-4) in F_1 triploid hybrids between Asiatic and African cottons shown in the table on the next page.



1. A bridge and a fragment at anaphase I ($\times 2375$)



FIG. 2. A bridge at metaphase II ($\times 2750$)



3. Three bridges at metaphase II ($\times 2375$) (all chromosomes not shown)

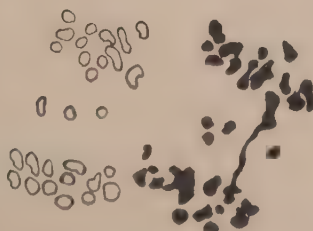


FIG. 4. A bridge and a fragment at anaphase II ($\times 2750$)

This research is being financed from the funds of the Indian Central Cotton Com-

Hybrid No.	Parents	
	American	Asiatic
S 29-1 . .	<i>G. barbadense</i> L (Sea Island)	<i>G. herbaceum</i> var. <i>frutes</i> (Surat 1027 A L F)—Delile
S 31-3 . .	"	"
S 38-1 . .	"	"
S 37-1 . .	<i>G. hirsutum</i> L (Coimbatore 2)	"

These bridges are clearly seen in acetocarmine smears of the flower at metaphase II (Figs. 2 and 3). The number of such bridges at this stage varied from one to four per nucleus, indicating that structural changes had taken place in more than one bivalent. The bridges are very similar to those figured by Miduno [1940] in the orchids and Srinath [1940] in the genus *Calceolaria*.

The formation of bridges at anaphase have been reported by Beasley [1940] in the F1 hybrid between *G. thurberi* Tod. \times *G. arboreum* var. *neglectum* H. & G., and by Ramiah and Gadkari [1941] in a sterile mutant of a strain of Asiatic cotton (*G. arboreum* var. *neglectum* forma *burmanica* H. & Beasley [1940] has also pointed out that such bridge formations give evidence of structural differences between the chromosomes of the species involved in his cross. That definite structural changes, like inversions and translocations could have taken place in cotton has been pointed out by J. B. [1941], where he shows one of the two chromosomes with a lateral satellite at the root-tip of *G. herbaceum* and *G. arboreum* and a ring chromosome in the root-tip of *G. herbaceum* var. *africanum* H. & G., at the metaphase stage. Further evidences that definite structural differences do exist between the chromosomes of certain species of cotton are pointed out in this note.

The formation of bridges may be due to several causes as inversions, translocations and duplications [Richardson, 1936]. A critical analysis of both metaphase and anaphase at division I is necessary to enable us to know what conditions have given rise to observed results and which of the structural changes that are possible have taken place. In the hybrids that are examined in the present study no abnormal configurations at metaphase I, as univalents, bivalents etc. could be clearly made out. The bridges that are seen at metaphase II are long and thin and their persistence at this stage shows that the bridges formed at anaphase I are not broken. The fragments that arise when such bridges are formed are difficult to make out in all the cases. The formation of a bridge at anaphase II, in one of the sister cells (Fig. 4), undoubtedly indicates that a loop chromatid must have been formed at anaphase I, as a result of an inversion pairing and two cross-overs having taken place, one in the inversion region and one in the region proximal to it, in which only one chromatid is involved in both the cross-overs. A monocentric loop and a fragment would be formed at anaphase I. The loop chromatid forms a bridge at metaphase II, the centromere having divided.

The above points indicate that in the triploids under study, we not only have numerical changes but structural changes as well. Both these factors may contribute to the sterility of the hybrids. All the same structural changes lead to the formation of new chromosomes which may be of evolutionary significance.

A fuller discussion of the above points will be published in due course.

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PLANT QUARANTINE NOTIFICATIONS

NOTICE No. 2 of 1942

INDIA

THE following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

I. U. S. Department of Agriculture, B. E. & P. Q.

1. *Summaries of plant quarantine import restrictions.*

(i) Republic of Cuba—Revision of regulations.

(ii) Republic of Uruguay—Standards established for Certain seeds.

(iii) Br. Colony of Malta—Area quarantined on account of Colorado Potato Beetle increased.

2. *Service and Regulatory Announcements.*

List—October—December 1941.

II. Other Announcements.

Jamaica—Br. W. I. : Citrus fruits in ports.

Notification No. 69/C. No. 429-Cus. II/41, dated 20 December 1941 of the Government of India in the Department of Finance (Central Revenues)

IN exercise of the powers conferred by section 19 of the Sea Customs Act, 1878 (VIII of 1878), the Central Government is pleased to prohibit with effect from 1 April 1942, the bringing into British India of bees or silk worms save where they are accompanied by—

(a) a special permit in accordance with the form set forth in the Schedule hereto annexed authorizing such importation issued by the Central Government or by an officer authorized by the Central Government in this behalf and

(b) a certificate of freedom from disease granted by an Entomologist of the Government of the country of origin.

SCHEDULE

Form of special permit authorizing importation of bees or silk-worms

1. Name, designation and full address of the importer
2. Name of the species of bees or silk-worms to be imported
3. Stage or stages of the bees or silk-worms to be imported
4. Country from which importation is sought

Whether importation is intended by sea, land or air

Name, designation and address of the exporter

Quantity indented for

Purpose of importation

The above information is true to the best of my belief

ate

(Signature of the importer)

I authorize the importation. This permit will be valid up to

ate

(Signature and designation of the certifying authority)

B. It is expected that the permit will be obtained in advance of sending the material so that the imported material may not remain indefinitely in the warehouse for a suitable permit.]

Notification No. F. 15-21/41-A., dated 12 May 1941 of the Government of India in the Department of Education, Health and Lands

Exercise of the powers conferred by sections 4A and 4D of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is directed to direct that the following further amendments shall be made in the notification of the Government of India in the Department of Education, Health and Lands, No.F.50-13 (20) /39-A, dated 20 November 1940, and the published therewith, namely:—

- I. In the preamble to the said notification, and in rule 1 of the said rules, after the word 'Punjab', the words 'the United Provinces' shall be inserted.
- II. In the Note below the Schedule annexed to the said rules, clauses (b) and (c) shall be re-lettered as clauses (c) and (d) respectively and before clause (c) as so relettered, the following clause shall be inserted, namely:—
'(b) in the United Provinces, by the Entomologist to the Government of the United Provinces, or such other officer as may be authorised by the Provincial Government in this behalf'.

GN

No. 1 of 1942 regarding plant quarantine regulations and import restrictions received in the Imperial Council of Agricultural Research

The following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those listed are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

LIST OF UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, IMPORT RESTRICTIONS, SERVICE REGULATORY AND OTHER ANNOUNCEMENTS

1. *Summaries of plant quarantine import restrictions.*—

- (i) Republic of Mexico.—Substitution of quarantines regarding coffee & banana
- (ii) Republic of Uruguay.—Restriction on the importation of seed potatoes
- (iii) Union of South Africa.—Revision of regulations concerning tomato seed
- (iv) British Colony of Bermuda.—Amendment of banana prohibition
- (v) Colony of British Guiana.—Restrictions of coffee and paddy & prohibition of citrus

2. *Service and Regulatory Announcements.*—

- (i) List—April—June 1941
- (ii) Index—1940

3. *Other announcements.*—

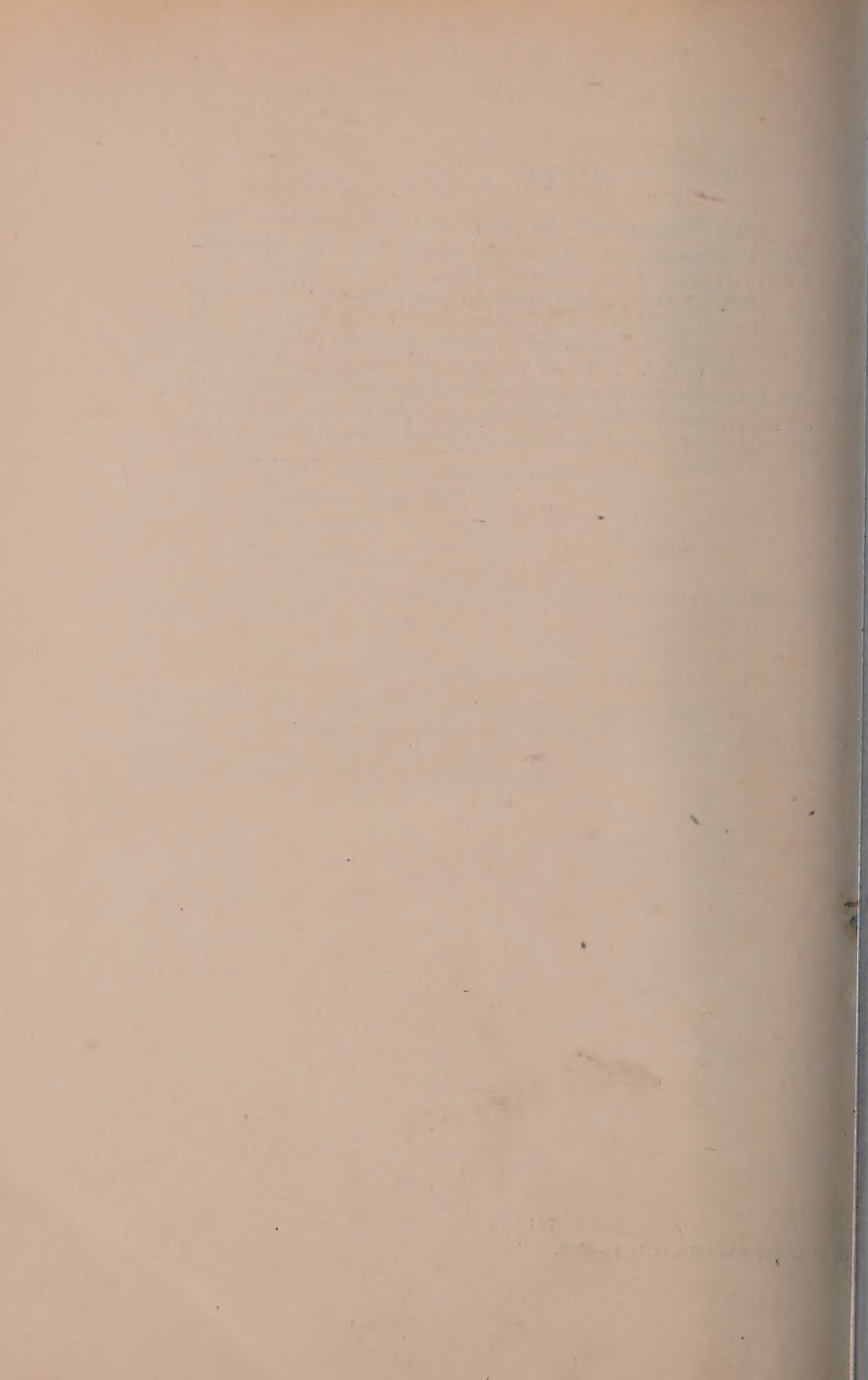
Canadian Order.—in council enabling the Inspectors to withhold certificates of inspection.

Exports of potato to Mauritius

IT is notified for general information that exports of potato to Mauritius must be accompanied by a certificate stating that the potatoes have been grown in a locality free from Potato Wart (*Synchytrium edoiceloticum*) and Colorado Beetle (*Leptinotarsa decemlineata*).

NOTE

an article entitled 'Studies on the Formation of Jellies from some Fruits' by Birendra Narain Singh and Sikhibhushan Dutt published in the *Indian Journal of Agricultural Science*, Vol. XI, Part VI, December 1941, pp. 1006-21, the authors claim to have discovered the jelly-forming qualities of wood apple in the following words :—'It has, however, been found by the present investigators to be an excellent jelly-forming fruit, being very rich both in acid and pectin.' It has now been brought to notice that the making of an effective wood apple jelly was first studied by S. S. Bhat, Horticulturist to Government, Baroda, at the Baroda Preservation Laboratory and the results published in *Rural India*, Vol. 3, March 1940 (Editor).



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TRICHINOPOLY FORT—Krishnaswami & Co., Messrs
S., Teppakulam.

VELLORE—Venkatasubban, Mr. A., Law Bookseller.

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(October 1942)

The Editorial Committee of the Imperial Council of Agricultural Research India, takes no responsibility for the opinions expressed in this Journal.

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Research Note—

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